MEDICAL PROGRESS

FAT TRANSPORT IN LIPOPROTEINS – AN INTEGRATED APPROACH TO MECHANISMS AND DISORDERS*

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THE subjects of this review are the plasma lipoproteins, their structure and functions and the ways in which they are disordered in certain diseases. The intent is not to discuss lipoproteins for their own sake, however, but to exploit their potential for illuminating the common and often frustrating clinical problem of hyperlipidemia. The finding of an abnormal concentration in plasma of cholesterol, glycerides or a given class of the lipoproteins often raises questions of cause and relief that have no certain answer. These will not necessarily be forthcoming in this report. What will be attempted is the reduction of current information about fat transport and metabolism to the minimum terms needed by a physician to obtain a rational approach to the patient with hyperlipidemia and to keep abreast of new developments in this rapidly expanding field.

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The integration of information and concepts about normal mechanisms and clinical disorders will proceed from more theoretical to more practical grounds. The first part of the review will outline the normal tasks of fat transport and describe how the several plasma lipids and certain proteins interact in their performances. The proteins that have evolved mainly to participate in transport of esterified lipids and the lipoproteins that they form will be closely examined. This will include analysis of several inheritable diseases in which one of these proteins is deficient to gain perspective on the functions that they apparently serve.

A detailed discussion of hyperlipidemia will follow. This will be based on an approach developed primarily for the study of genetically determined abnormalities, but acquired or nonfamilial disorders, including changes in lipid concentrations secondary to other known disease, will be dealt with as well. All these disorders are translated into *hyperlipoproteinemia* on the premise – for which supporting evidence will be presented – that lipoprotein patterns offer necessary information not provided by analyses of plasma lipids alone. Some simple new nomenclature is offered since the older terminology

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obscures the heterogeneity that has recently been discovered. Attention will also be paid to practical steps to diagnosis and conservative therapy suitable for the great majority of patients with hyperlipoproteinemia.

It will be necessary to cover the enormous literature in the area of this report in a selective rather than a comprehensive fashion. Whenever possible general references will be introduced with major topics and the number of specific citations held to a minimum.

The Normal Fat-Transport Tasks[1-4]

It seems wisest to begin by considering the kinds and amounts of lipids that move through the extracellular waterways. Most of the lipid in plasma at any one time is usually not in rapid transit from one tissue to another. Cholesterol and phospholipids, which represent about two thirds of the plasma lipid, have a much slower turnover than fatty acids. In quantitative terms the major fat-transport tasks are movement of free fatty acids and fatty acid esters of glycerol (glycerides). The lipoprotein concentrations in plasma are directly and indirectly influenced by this traffic, which in turn depends on many factors, an important one of which is the state of carbohydrate metabolism.

Free Fatty Acids

By far the greatest amount of fat transported through the plasma compartment is in the form of free fatty acids. This is despite their relatively insignificant contribution to the total plasma lipid concentration. In the fasting subject, there are from 0.3 to 0.7 mEq. per liter of plasma, about 8 to 20 mg. of the total lipid concentration of 400 to 800 mg. per 100 ml. The content of individual free fatty acids is similar but not identical to that of adipose-tissue triglycerides, from which they mainly originate. These fatty acids do not actually circulate in the "free" state but bound to albumin. The free-fatty-acid-albumin complex is formed immediately when fatty acids are released into the bloodstream and the complex releases its fatty acid at sites of utilization, which include liver, muscle, heart and many other tissues. This process is under sensitive metabolic control that is rapidly adjusted to meet the body's ever-changing need for metabolic fuel. Release of free fatty acids depends acutely on the availability of insulin, but is further adjusted by the sympathetic nervous system and by circulating catecholamines; adrenocortical hormones, thyroid hormone, glucagon and several anterior pituitary hormones also have permissive or secondary roles in mobilization of free fatty acids.

In the postabsorptive state from 50 to 90 per cent of the body's total energy needs are met by free fatty acids delivered from the adipose tissue. More than 25 gm. per hour may be transported through plasma during the day although much of this is re-esterified rather than oxidized immediately. The major direct contribution of the free fatty acids to plasma lipid concentrations is their conversion in the liver to glycerides. When flux of free fatty acids to the liver is unusually high, in considerable excess of the ability of that organ to burn them, the outpouring of such glyceride can be great, leading to a form of "endogenous hyperlipemia." Although the importance of free fatty acid transport cannot be overemphasized it would be tangential to our purposes to attempt to cover many details available in a number of reviews.[1-4] Free fatty acids will be mentioned further only when directly pertinent to lipoprotein metabolism and disorders thereof.

Glyceride Transport

Exogenous. From the first days of life a second major task must be performed, the disposal of glycerides that are ingested in the amount of 1 to 2 gm. per kilogram of body weight daily. These are hydrolyzed in the intestinal lumen and taken up along with smaller amounts of other lipids and lipid-soluble substances. In the mucosal cells glycerides are reformed and collected into particles. (The term particle is conventionally applied to circulating lipid-protein complexes large enough to be seen in the light microscope – that is, about 0.1 μ or larger.[5]) The chylomicrons or "exogenous particles" formed in the intestinal cells during fat absorption are released into intestinal lymphatics and enter the bloodstream through the thoracic duct. Either at the capillary surface or immediately on entering cells in adipose tissue, liver, heart and other organs, the chylomicron glycerides are hydrolyzed, and the constituent fatty acids reformed into other esters within the cell.

Endogenous. The plasma of normal fasting subjects contains glyceride in concentrations of 10 to 190 mg. per 100 ml. This glyceride, which appears for the most part to be synthesized in the liver, is in the form of very-low-density lipoproteins that will be called pre-β in this review. If the concentration of such glyceride is abnormally increased the pre-β lipoproteins become larger or particulate in size. Such "endogenous particles" differ from chylomicrons not only in their origin but also in their physical properties and their content of cholesterol, phospholipid and glyceride.[6,7]

The turnover of glycerides in pre-β lipoproteins seems to be slower than that in chylomicrons. This is more probably due to their smaller average size[6,7] than to any chemical differences, since it has been suggested that the clearing rate of particles is proportional to size.[8] At ordinary plasma concentrations the rate of removal has been calculated to be about 2 gm. per hour.[9] This estimate is subject to a number of qualifications and possibly much variation, but its relatively low order of magnitude implies that factors that accelerate synthesis and secretion of these lipoproteins may rapidly produce endogenous hyperlipemia.

Minor Transport Tasks

Cholesterol Transport[10-14]

In contrast to the large amounts of free fatty acids and glyceride that must be transported through the plasma compartment each day, the net movement of cholesterol between tissues appears to be small indeed. From 100 to 500 mg. of cholesterol per day is usually absorbed from the diet, to which is added roughly another gram of sterol resorbed from the intestinal lumen upon its secretion from bile or intestinal mucosa. Most tissues are capable of cholesterol synthesis, and none has been known to require that cholesterol be transported to it via the plasma to meet its demands, although this may be occurring, for example, in organs using cholesterol as a precursor for hormone synthesis.

An unknown amount of cholesterol must be retransported to the liver to take advantage of this organ's unique capability to degrade sterol to bile acids, which may then be excreted. The circulating red blood cells, for example, represent a total pool of about 4 gm. of cholesterol. With the replacement of 1 per cent of this mass per day about 40 mg. of cholesterol must be transferred from sites where red cells are broken down to where they are made, or the cholesterol must be catabolized. The total of all such anabolic and catabolic processes is a daily body turnover of cholesterol approximating 1 gm.[10,11]

Cholesterol coming in from the intestine does so in association with chylomicrons, and much of this seems to disembark in the liver. From this point the exogenous cholesterol, like that which is made endogenously, is usually carried from tissue to tissue in the form of α and β lipoproteins. There is rapid exchange of free cholesterol between the various transport forms and many tissues, most swiftly between plasma, liver and red blood cells; the resultant randomization of molecules makes any calculation of net transport from tracer studies most difficult. For present purposes, the important conclusion to be drawn is that most of the cholesterol in plasma is not earmarked as cargo but is there for another purpose, as a structural component of lipoproteins, vehicles for transport of other lipid.

Phospholipid Transport

The phospholipids, which in plasma are mostly phosphatidyl choline and sphingomyelin,[15] exceed all other lipid classes in their contribution to the total mass of lipids. If these molecules have a role in carrying specific fatty acids between tissues it has thus far been a silent one. The total plasma phospholipid pool is estimated to turn over about every three days in man.[16] It is possible to reach erroneous conclusions about the turnover of phospholipids if they are considered as a single pool, for many molecular species, differing in fatty acid content, are represented. One cannot escape the intuitive conclusion, however, that the phospholipids, always predictable in their composition and varying only sluggishly in concentration, are mainly in plasma to function as "biologic detergents." Their high surface activity promotes stability at the oil-water interfaces represented by the lipoproteins and their interactions with plasma.

Carotenoids and Fat-Soluble Vitamins[17-21]

The diet contains several milligrams of various carotenoids per day. These pigments are of importance to man because some of them are convertible in the intestine into vitamin A. This essential fat-soluble vitamin is present in the diet in only microgram quantities. During digestion, vitamin A alcohol (retinol) and ester, formed in the gut from β carotene, are transported along with unchanged carotene into the bloodstream in association with lymph chylomicrons. In the postabsorptive state the carotenes are found mainly in the β lipoproteins whereas vitamin A (retinol) is associated with still unidentified proteins of density greater than the lipoproteins (more than 1.21).

Vitamin E is also transported from the intestine in the chylomicron lipids, and carried in the blood predominantly with the β lipoproteins. Little is known about the transport of vitamins D and K, but these too are probably adsorbed to the chylomicrons and carried on the β lipoproteins in the postprandial state.

To summarize the known demands for lipid transport in man and quite likely many other species, the movement of fatty acids for maintenance of "caloric homeostasis" far overshadows all other requirements. Food intake gives rise to several tidal waves of exogenous glyceride. As the last of these ebbs, and the delivery of dietary glucose declines as well, an increase in movement of free fatty acids out from the adipose tissues occurs. Some of these fatty acids and carbohydrates are converted to endogenous glycerides. The ones that the liver cannot store are sent back to the adipose tissue. The interactions of these several transport circuits and their responses to changing demands will crop up frequently as this discussion proceeds, for they are the basis for several forms of hyperlipoproteinemia.

In considering the manner in which lipids combine with proteins to serve the major transport tasks, we shall, again, only mention the important transport pathway for free fatty acids. Albumin is uniquely involved, and the protein-lipid complex formed is neither considered a lipoprotein nor measured in the usual quantification of lipids or lipoproteins. When albumin is deficient or free fatty acid concentrations are unusually high free fatty acids may travel with lipoproteins and affect the lipoprotein patterns, but these are special cases rarely encountered. The lipoproteins, on the other hand, are the keys to a more rational approach to hyperlipidemia, and the proteins involved and their various combinations with lipids will now be examined in some detail.

The Plasma Lipoproteins[1,5,22-24]

None of the plasma lipids are sufficiently polar to circulate free in solution. They depend upon interactions with protein, and the resulting "macromolecules" or "micromicelles" are referred to by the generic term of lipoproteins. There is a tendency to restrict the term "lipoprotein" to the soluble complexes and to use the name "particle" for those that scatter light and approach a 2-phase distribution of lipid and plasma as they get large. For purposes of simplification the concept will be adopted here that there are 2 basic kinds of lipoproteins, the α and the β, and that these act to solubilize varying amounts of glyceride. Followed to its logical conclusion, this means that particles therefore contain lipoproteins. The advantages of this point of view, which has not been strictly proved to be true, will emerge presently.

There is much uncertainty about the structural relations of the lipid to the proteins in these macromolecules. It remains to be proved for any of the lipoprotein forms whether the protein serves as a film over the surface of all the lipid, exists as a central core or is sandwiched between alternate lipid moieties. The nature of the bonds between lipid and protein in the lipoproteins is also speculative. Few are covalent.[25] They are strong enough to resist dissociation during the physical processes used to isolate the lipoproteins; yet they allow for the exchange of lipid between plasma lipoproteins themselves and between tissue and plasma lipoproteins.

Isolation[1,26,27]

There are many methods for isolating and characterizing lipoproteins. These take advantage of the fact that the lipoproteins behave as euglobulins but have physical properties that are determined by their content of both protein and lipids and permit separation by methods as diverse as salting out, ethanol-salt fractionation,[28] precipitation by antibodies and nonspecific polyanions,[29] electrophoresis,[30-32] ultracentrifugation[33,34] and chromatography.[35-38]

Lipoproteins isolated by other technics are invariably equated with lipoproteins prepared by the 2 most widely used methods, ultracentrifugation and electrophoresis, which have the greatest range and adaptability. The analytical ultracentrifuge, which can quantitatively determine an almost limitless number of subgroups (S_f classes) of lipoproteins varying by small increments of differences in their densities, has capabilities beyond the current requirements of many experimental or clinical studies.[39]

The groups of lipoproteins important in clinical work are 4: high-density or α lipoproteins; low-density or β lipoproteins; very-low-density or pre-β- (also called α_2-) lipoproteins; and chylomicrons. Although everyone agrees with this subdivision as far as it goes there is not complete accord over how these groups of lipoproteins are related to each other or whether they contain subgroups that also have independent metabolic behavior.

This uncertainty is moving toward resolution through wider employment of immunochemical standards for comparing the content and measuring the purity of lipoproteins isolated by different procedures. The lipoproteins are good antigens. Their specificity devolves from the protein moiety, the lipids contributing in only a minor way as haptenes. The search for immunochemical determinants has exposed the presence of several different proteins and greatly enhanced the understanding of lipoproteins in general.

The Lipoprotein Apoproteins

Two different proteins are consistently isolated from plasma lipoproteins. A third has been found in 1 group of lipoproteins. These proteins, which some favor calling apoproteins in the lipid-free state, are generally designated as A (or α), B (or β) and C proteins. They differ in their terminal residues, total amino acid content and immunochemical behavior. The presence of 1 or both of the A and B proteins, combined with lipid, accounts for the known functions and chemical properties of all the plasma lipoproteins with one exception. This is the frequent demonstration of aminoterminal serine and threonine in proteins obtained from very-low-density lipoproteins, attributed by some to the C protein.

Normally, the A protein is the only protein found in the α_1-migrating (high-density) lipoproteins and the B protein in the β-migrating (low-density) lipoproteins. In some diseases the normal distribution of either protein is distorted. For example, A protein appears in the low-density lipoproteins in obstructive liver disease and in the rare disease, abetalipoproteinemia. At times the B protein appears in the high-density lipoproteins; most commonly this implies that the manipulations involved in lipoprotein isolation have been too severe. Both proteins can be isolated in the very-low-density lipoproteins and chylomicrons.

The appearance of A and B proteins individually in the 2 soluble lipoproteins and together in the larger glyceride-rich particles is the basis for the major simplification of lipoprotein metabolism adopted for this review (Fig. 1). This concept[40] is simplistic and vulnerable to a number of possible contradictions as knowledge of lipoproteins unfolds. In brief, it assumes that A and B proteins are the primary components of the lipoproteins. In plasma they usually occur with predictable complements of lipid that feature differing proportions of cholesterol and phospholipids. When glycerides appear in quantity these 2 lipoproteins become involved with its transport. Glyceride (Fig. 1) thus becomes the third and most dynamic factor in determining the nature of the lipoprotein distribution in plasma.

Alpha Lipoprotein

A crude α lipoprotein was isolated from horse serum in 1929 by Macheboeuf,[41] who used salt precipitation in a way somewhat similar to the later

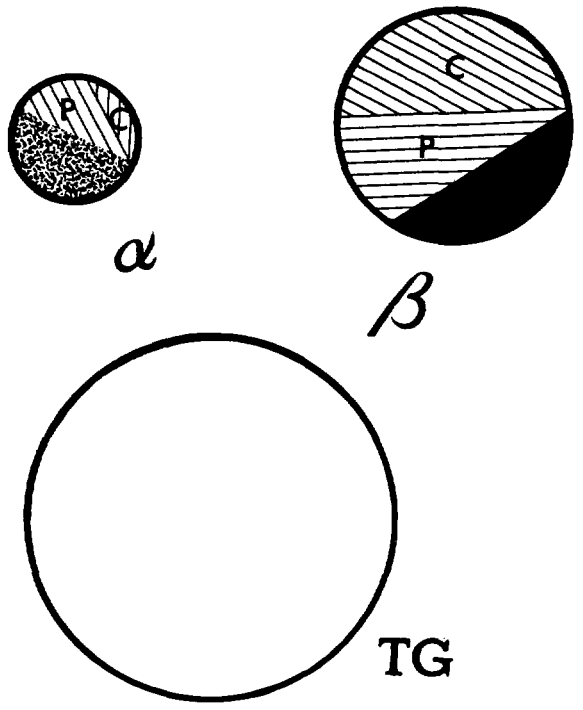

FIGURE 1. *Symbols for the 3 Most Important Factors in the Simplest Concept of Fat Transport by Lipoproteins — α = Alpha Lipoprotein, β = Beta Lipoprotein; TG = Triglyceride; the Phospholipid (P), Cholesterol (C) and Protein (Stippled for A Apoprotein and Solid for B Apoprotein) — Allocated Areas in the Symbols Comparable to Their Contribution by Weight to the Lipoproteins. In all subsequent figures the protein moiety only will be shown in the circular lipoprotein symbols.*

Cohn fractionation method.[28] The Cohn fraction IV-1 contains lipoproteins that have α_1 mobility on either free, starch or paper electrophoresis and correspond to the "high-density" lipoproteins isolated in the ultracentrifuge between the densities of 1.063 and 1.21. Alpha lipoproteins are normally not precipitated by polyanions like heparin that aggregate all the low-density lipoproteins and are thus the only lipoproteins remaining in the supernatant after such treatment of plasma. They have the highest content of phospholipids and protein of all the lipoproteins. They are relatively stable and can be delipidated with solvents to yield a water-soluble A protein containing only a little phospholipid. The physical and chemical properties of the α lipoproteins are better known than are those of the lower-density lipoproteins. The metabolism of the latter has been far better studied, however, owing in part to the interest generated by their frequent indictment for complicity in causing atherosclerosis.

Inferences from what little is known of the comparative biochemistry of lipoproteins suggests that on the evolutionary scale the α lipoproteins may be the older of the 2 major soluble lipoproteins. In many mammals (when not hibernating) the bulk of the lipid content in the postabsorptive plasma is present in α lipoproteins.[34] In man and other primates the total weight and lipid content of the β lipoproteins is greater. Nevertheless, in human plasma there is still much more A protein than B (approximately 140 mg. of the former as compared to 80 mg. of the latter per 100 ml.).

Neither Cohn fractionation, electrophoresis nor polyanion precipitation alone provides α lipoproteins uncontaminated by other serum proteins. Preparative ultracentrifugation must be employed when it is desired to isolate a large quantity of material as nearly pure as possible. In our experience, the highest yields are obtained by ultracentrifugation of plasma that has been brought to a density of 1.21 by the addition of salt. The supernatant is washed free of nonlipoprotein material by ultracentrifugation repeated once or, at most, twice. All the low-density lipoproteins are then precipitated with heparin and manganese.[42] Alpha lipoproteins isolated in this way ideally should contain only A protein. As determined immunochemically, however, traces of B protein are not uncommonly present.

The A Protein[22,27]

Pure α lipoproteins can be extracted to obtain the A protein. Several stages of delipidation are possible. Exposure of the lipoproteins to cold ether alone extracts only some of the cholesterol and fails to remove any of the phospholipids. Pretreatment of the lipoproteins with trypsin, chymotrypsin, or phospholipases increases the amount of lipids extracted by ether. Delipidation of lyophilized α lipoproteins in the presence of starch granules[43] removes all the neutral lipids and leaves a phospholipid protein that is quite stable and may represent an important structural unit or stage in lipoprotein formation. The best method to date for obtaining A protein that is water soluble is prolonged treatment of α lipoproteins with ethanol and ether in the cold. The product still contains a small amount of tightly bound phospholipids.

The essentially lipid-free A protein obtained by ethanol-ether extraction still contains over 3 per cent carbohydrate by weight.[44,45] The B protein isolated from β lipoproteins similarly contains carbohydrates.[46] The total amount of carbohydrates present in the lipoproteins before delipidation, their exact chemical form and whatever function they are serving remain to be determined.

The A protein obtained in this fashion seems to be a polymer or aggregate whose components readily separate and recombine in response to changes in pH, ionic strength and the presence or absence of urea or detergents like sodium dodecyl sulfate.[27,47,48] The protein may consist of identical subunits whose molecular weight has been estimated to be between 23,000 and 36,000.[48,49] Upon exposure of the A protein to depolymerizing agents, the polymer of $S_{20w}4.5$ yields several units of $S_{20w}2.3$. These units reaggregate rapidly in solution. It is believed that the A protein in native α lipoprotein represents a polymer of 2 to 6 units.

These subunits contain aminoterminal aspartic

acid and carboxyterminal threonine. In the amino acid analyzer their composition is indistinguishable. They have large amounts of glutamic acid and leucine and small amounts (1 or 2 moles per mole of protein) of isoleucine, methionine and cystine.[49,50]

Upon electrophoresis in either agar or agarose the A protein migrates more slowly than the native α lipoprotein. On starch or Pevikon this difference is reversed. The A protein appears to have a high degree of helical structural organization whether lipid is present or not, suggesting that the tertiary structure of the protein is independent of its interactions with lipid.[51]

The A protein reacts with antiserums against α lipoproteins. When ^{131}I-labeled A protein is introduced into plasma, it disappears at the same rate as α lipoprotein.[27,52] The A protein has great avidity for lipid. Whenever it is exposed to lipid suspensions in vitro or to other lipoproteins nearly all of it is recovered with the lipid or lipoproteins.

Alpha Lipoproteins[53-55]

There has not yet been convincing proof that A protein is present in plasma except as α lipoprotein. When plasma is ultracentrifuged at density 1.21 the lipoprotein-poor infranate usually yields a small amount of immunoprecipitate with anti-α-lipoprotein serum. There is no certainty that this represents α lipoprotein or A protein that was present in the native state because the process of ultracentrifugation delipidates some α lipoproteins and the products of this transformation also sediment at density 1.21.[50] Recently, an "apoprotein" capable of recombining with lipid has been described in similar preparations (density greater than 1.21 infranatant fractions) from rat plasma.[56] This protein has not yet been shown to be A (or B) protein.

The lipid and protein sedimenting at density greater than 1.21, sometimes collectively called "very-high-density lipoproteins," still remain uncertainly related to the rest of the plasma lipoproteins. This fraction accounts for about 8 per cent of the plasma phospholipids and includes trace amounts of neutral lipid. Most of the phospholipids are lysolecithins.[57] It has been proposed these may be the product of a plasma enzyme transferring a fatty acid molecule from lecithin to esterify cholesterol.[58] The lysolecithin may be complexed with albumin,[59] but further experimental confirmation is needed.

Normally most of the α lipoproteins can be isolated from plasma between the densities of 1.063 and 1.21. This is a rather broad density band, which can be further subdivided in the ultracentrifuge.[33] The heavier α-lipoprotein fractions contain relatively more A protein and phospholipid and less cholesterol than the lighter ones. The "average" α lipoproteins are composed (in dry weight) of 45 to 55 per cent protein, about 30 per cent phospholipid and about 18 per cent cholesterol. Five sixths of the latter is esterified. Small amounts of glyceride are also found in most preparations. The hydrated lipoproteins contain about 15 per cent water. Estimates of molecular weight vary from 165,000 to 400,000.[27,49,60] As determined by light-scattering and viscometry the lipoprotein seems to be a prolate ellipsoid about 300 × 50 Å.[60,61]

Compared to the β lipoproteins, the α lipoproteins contain relatively more esterified cholesterol and phospholipids. The fatty acid patterns of the different lipid moieties are similar in the 2 lipoproteins.[62] The ratio (by weight) of sphingomyelin to lecithin in α lipoproteins is about 0.2.[63]

Immunochemistry[50]

The immunochemistry of the plasma lipoproteins is summarized in Figure 2. The α lipoproteins and their A protein are less antigenic than the larger and more lipid-rich β lipoproteins. When the latter are present even in trace amounts they stimulate the production of a potent anti-β-lipoprotein serum. This is a persistent problem in preparation of antiserums to α lipoproteins. As previously indicated, antiserum prepared to either the A protein or the α lipoprotein usually crossreact. Only 1 immunologic form of α lipoprotein is usually detectable in fresh plasma. At least 1 other antigenic form becomes readily detectable after ultracentrifugation, freeze thawing or brief storage at room temperature. These 2 α lipoprotein antigens are only partially crossreactive with most antiserums. We have designated the native α lipoprotein as "αLP_A." This is represented by the forward migrating α-precipitation line in Figure 2. The slower migrating antigen (αLP_B) is partially delipidated α lipoprotein believed to contain a smaller polymer of the basic subunit of A protein. Only αLP_A is present in the high-density lipoproteins isolated between density 1.063 and 1.12 (called the HDL_2 subclass when it is quantified in the analytical ultracentrifuge).[33] Both αLP_A and αLP_B are present in the subclass separated in the ultracentrifuge between densities 1.12 and 1.21 (called HDL_3 in the analytical ultracentrifuge). Since αLP_B is formed during ultracentrifugation it has been suggested that the relative concentrations of HDL_2 and HDL_3 may represent the degree to which native α lipoproteins are dissociated in the ultracentrifuge.[50] The plasma concentrations of these 2 "forms" of α lipoproteins vary greatly with certain metabolic disorders as they are measured in the analytical ultracentrifuge.[64] It is possible that this is a function not only of the degree of dissociability of the lipoproteins but also of other factors still unappreciated that may control the amount of lipid associated with the A protein or its state of polymerization. Other small peaks have been seen in the analytical ultracentrifuge besides the HDL_2 and HDL_3 fractions.[65] The possibility that these are technical artifacts has been raised.[66]

Beta Lipoproteins

The Lipoprotein

The β lipoproteins represent a second homogene-

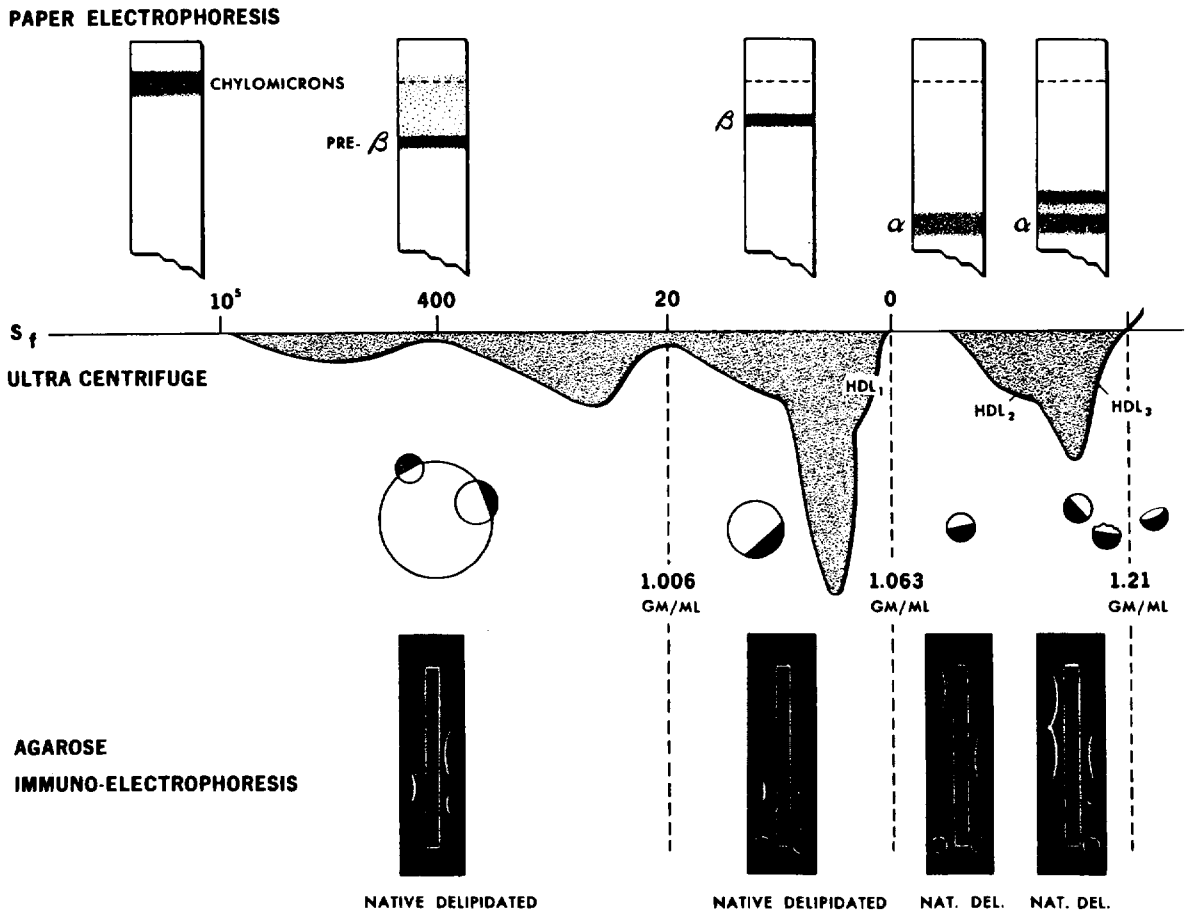

FIGURE 2. *Schematic Representation of the Major Portions of the Lipoprotein Spectrum as Defined by Paper Electrophoresis, the Ultracentrifuge and by Immunoelectrophoresis Using Antiserums Reacting with Both α and β Lipoproteins. The protein content is also depicted as in Figure 1.*

ous component of the plasma lipoproteins. In the newborn infant practically all the plasma lipid not present in α lipoproteins is found in β lipoproteins.[67] This perhaps "ideal" condition also holds in most healthy, actively growing young human beings in the postabsorptive state. As the members of most populations grow older, however, there is a progressive departure from this simple picture of plasma lipoproteins.

Beta lipoproteins migrate with sharp boundaries in the β zone on most types of electrophoresis and can be isolated in bulk in the Cohn fraction III-0. In the ultracentrifuge β lipoproteins are isolated between the densities 1.006 and 1.063 and have a mean density of about 1.03.[33] Like the α lipoproteins, β lipoproteins are found at densities less than 1.006 when plasma glyceride concentrations rise. In these cases they do not *usually* have their β mobility but are part of the pre-β lipoproteins and chylomicrons. In the analytical ultracentrifuge, according to the technic of Gofman et al.,[33,64] the pure β lipoproteins normally are in the S_f subclass of 0-20; they have a mean S_f of 6 and are mostly in the subclass S_f 0-12. (S_f refers to Svedberg units of flotation expressed in 10^{-13} cm. per second per dyne per gram).

In man β lipoproteins are the major cholesterol-bearing lipoproteins. In the dry state a "typical" β lipoprotein consists (by weight) of 20 to 25 per cent protein, 8 per cent free and 35 per cent esterified cholesterol, 22 per cent phospholipid and 10 per cent triglyceride.[68] In its native state it is extensively hydrated. The fatty acid components of its constituent lipids are similar to those found in the total plasma lipids and in α lipoproteins.[53] There is relatively more sphingomyelin in the β lipoproteins than in α, ratio of the sphingomyelin to lecithin being about 0.4.[63]

The molecular weight of representative β lipoproteins has been estimated at anywhere from 1.3 to 3.2×10^6.[61,69] Light-scattering studies indicate that the usual complex is dyssymetric and a prolate ellipsoid of about 150 × 350 Å.[61]

Mild treatment with cold ether or n-heptane readily removes much of the cholesterol and glycerides from the β lipoproteins.[22,27,43] Attempts to remove the neutral lipids more completely or to strip away phospholipids usually yield a gel-like product that

is irreversibly aggregated. Both the state of hydration and the presence of lipid appear to be very important in maintaining the integrity of β lipoproteins. Recently, delipidation in the presence of depolymerizing agents like urea and sodium dodecyl sulfate has shown promise of yielding a lipid-free and soluble protein from β lipoprotein.[70]

The B Protein[71-76]

The difficulties encountered in obtaining lipid-free B protein leave it less well characterized than the A protein. The low-density lipoproteins in egg yolk (lipovitellin) have been suggested as a model for β lipoprotein.[77] When these are delipidated stepwise formation of smaller protein components, each approximately half the size of its immediate precursor, occurs. The total number of polypeptide chains is partially dependent on the total lipid content of the complex. Analogous but less complete experiments with human β lipoproteins suggest that the B protein in the native lipoprotein consists of several identical or at least similar peptide chains. Two identical protein units of molecular weight 380,000 have been reported in lipoproteins having an S_f 7.9 flotation value. Other predictions of protein molecular weight have been as low as 250,000. Recent work suggests a protein on the order of 100,000 molecular weight as a possible repeating subunit.[76] The B protein contains aminoterminal glutamic acid, carboxyterminal serine and a total amino acid pattern that differs from the A protein particularly in the relative contents of isoleucine, leucine, glutamic acid and alanine.[27,50,74]

Immunochemistry[78,79]

Immunochemical studies of β lipoproteins are subject to difficulties not encountered with α owing to interactions with media commonly used for precipitation reactions. Precipitation lines may appear that do not reflect true immunoprecipitation. Purified agar and especially sulfate-free agar (agarose) have made it easier to work with β lipoproteins. In agarose they migrate in the β region and, although they still do not diffuse freely, usually give a sharp immunoprecipitation line with anti-β-lipoprotein serums (Fig. 2).

Most antiserums reacting with β lipoproteins also react with the soluble phospholipid-protein complex obtained by mild ether delipidation. Some antibodies fail to react with the products of more vigorous lipid extraction, suggesting that the neutral lipids of the lipoproteins are important as haptenes. It has not yet been shown that an anti-β-lipoprotein serum will react with a completely lipid-free B protein. This has made it impossible to learn whether B protein circulates in plasma or exists in tissues without appreciable amounts of associated lipid.

Thus far it appears that all β lipoproteins obtained from a given subject are antigenically homogeneous, but it is likely that as methods are improved antigenic forms of β lipoprotein sharing only partial identity will be detected in the same individual as is the case with α lipoproteins. Several variations (polymorphism) in plasma β lipoproteins have already been reported in man. These fall into 3 categories. In the first place, minor antigenic differences occur between the β lipoproteins in different subjects; these appear to be genetically determined.[80-84] They are usually demonstrable by immunochemical technics using antiserums obtained from patients who have had multiple blood transfusions. Secondly, there are cases in which β lipoproteins differ from the normal in their electrophoretic and ultracentrifugal behavior. A peculiar "broad" β lipoprotein found in Type III hyperlipoproteinemia will later be described in detail. There is also a recently described "double β-lipoprotein" anomaly first detected by starch-gel electrophoresis and restricted thus far to 1 family.[85] Here there appears to be an alteration in the physical state, perhaps in degree of polymerization, of some of the β lipoproteins, giving them greater density and a higher molecular weight than the normal. This intriguing abnormality may provide a means for learning more about what controls the normal structure of the lipoprotein. Finally, the β lipoproteins also contain enzyme activity, especially esterases.[86] It is not yet known whether this is located in the B protein or represents other proteins adsorbed to the β lipoprotein molecules.

Structure and Intracellular Metabolism of the Lipoproteins

No further attempt will be made here to review the fine structure of lipoproteins. The subject must lean heavily upon analogies drawn from colloidal and surface chemistry or study of bimolecular leaflets such as myelin, and little progress beyond the theoretical has been made. Since the plasma lipoproteins provide uniquely accessible models for approaching the structural features of other lipid-protein complexes in cells, knowledge in this area may be expected to increase rapidly.

When certain difficulties encountered in the study of tissue lipoproteins are overcome it will also be possible to learn more about how and where the plasma lipoproteins are synthesized and broken down. It is not easy to apply to tissues the technics presently used with plasma lipoproteins. Frequent contamination of tissues with plasma, lability of the relatively weak lipid-protein bonds and frequent exchange or adherence of labeled precursors to lipoproteins all complicate experiments dealing with cellular lipoproteins.

There is fairly good evidence that the liver is capable of synthesizing lipoproteins identical to those found in plasma. Incorporation of ^{14}C labeled amino acids, and apparently net synthesis of β lipoproteins (density less than 1.063), occurs in rat-liver slices and perfused rat livers.[87-89] The ability of the rat liver to make lipoproteins that correspond in peptide pattern to plasma α lipoproteins has been

similarly demonstrated,[90] and synthesis of lipoproteins has been reported in rat-liver microsomes.[91]

Less conclusive experiments suggest that intestinal-mucosa cells can incorporate labeled amino acids into both α and β lipoproteins.[92-94] This is in keeping with a recent report that the hepatectomized dog can still synthesize plasma lipoprotein,[95] but there yet is no basis for assessing the contribution of the intestine to the plasma lipoprotein pools.

Inhibitors of protein synthesis such as puromycin cause accumulation of lipid in both the intestinal and liver cells and therefore presumably inhibit either the synthesis or the release of plasma lipoproteins.[96,97] In the liver the same effect has been shown for hepatotoxins such as carbon tetrachloride. Orotic acid not only produces a fatty liver but eliminates nearly all the plasma β lipoprotein in rats, a nearly specific effect that is quickly reversed by adenine.[98,99]

Turnover of plasma α lipoproteins has been studied by labeling of the protein with ^{131}I. The biologic half-life of the protein, either in lipoprotein or as apoprotein is about four days.[27,52,100] A similar half-life has been obtained with β lipoprotein tagged with either ^{131}I or ^{35}S.[100,101] The plasma lipoprotein proteins thus turn over faster than most other major plasma proteins with the exception of fibrinogen.[101]

Recapitulation

Those who feel no great concern with the minutiae concerning the plasma lipoproteins may find it easiest to consider the essential elements as being 3, all represented symbolically in Figure 1. Two of these are lipoproteins. Each consists of a different protein, the A (or α) and the B (or β), that has unusual affinity for lipids and prefers to circulate in extracellular fluid accompanied by a complement of cholesterol and phospholipid. The lipoproteins that result have densities and electrostatic charges that differ sufficiently to permit their operational definition by several technics. By the most commonly employed methods, electrophoresis and the ultracentrifuge, they are identified as α, or high-density, lipoproteins and β, or low-density, lipoproteins.

The α and β lipoproteins normally account for about 90 per cent of the cholesterol and phospholipid in plasma. The concentrations of those lipids undergo very little tidal change in comparison to that of the other major group of neutral lipids found in plasma, the glycerides. Glycerides are the third element in any discussion of lipoproteins and fat transport (Fig. 1). They are the dynamic factor to which the α and β lipoproteins seem related as vehicles are to cargo. When appreciable glyceride is present in plasma it associates with both α and β lipoproteins in such a way as to decrease their density and alter the net effect of the charges on the lipoproteins. The resultant combinations differ, depending on whether the glycerides are coming in from the diet or are made in the body. This distinction is of great importance when one is in dealing with hyperlipoproteinemia and merits detailed consideration, which will be given in the next section of this review.

Glyceride Transport

Two Forms of Plasma Glyceride Transport

In subjects on fat-free diets the plasma lipid concentrations vary little throughout the day and tend to be highest just before breakfast.[102] When the diet contains fat the levels of cholesterol and phospholipids in plasma are still relatively constant, but the glyceride concentration, as already intimated, is quite variable. It is lowest during the early morning hours (when blood samples for analysis are usually taken) and rises sharply, reaching a peak about three hours after breakfast. The concentration is boosted again by the midday meal, and after some decline in the afternoon, again rises after dinner, ebbing during the night.

The form in which glycerides are transported in the plasma depends on whether their immediate source is dietary fat or endogenous synthesis (Fig. 3). In normal subjects before breakfast, and in those on fat-free diets at all times, the plasma glycerides are exclusively of endogenous origin and are carried predominantly on pre-β lipoproteins and particles. Glycerides from dietary fat, on the other hand, are carried on the chylomicrons.

Methods

Analytical methods for the detection and quantitation of α and β lipoproteins are relatively simple, and the technics employed are generally comparable from one laboratory to another. In the analysis of the glyceride-rich lipoproteins, however, there is no such uniformity. Many different procedures are used, none of them ideal. The ones that are of greatest value at present have one common feature, the ability to provide a qualitative separation of lipoproteins and particles carrying mainly endogenous glycerides from those containing exogenous

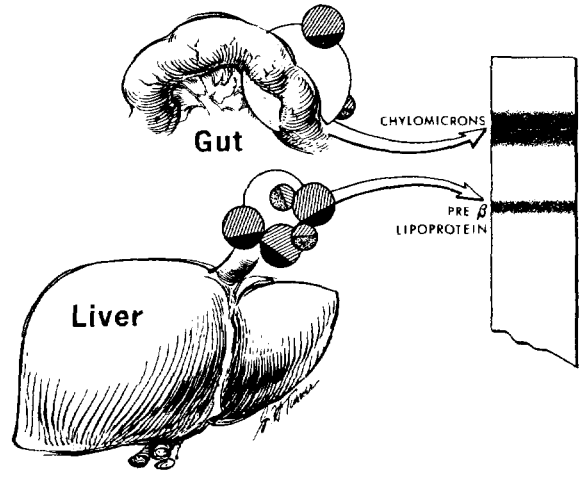

FIGURE 3. *Origins of Plasma Glycerides and the Lipoproteins in Which They Are Found as Separated by Paper Electrophoresis.*

TABLE 1. *Very-Low-Density Lipoproteins—Composition and Correlations.*

Band on Paper Electrophoresis	Migration in Starch-Block Electrophoresis	Location in Polyvinylpyrrolidone Density Gradient	Approximate S_f by Ultracentrifugation	Protein	Lipid		
					Glyceride	Cholesterol	Phospholipid
				% total weight	*% total lipid*	*% total lipid*	*% total lipid*
Chylomicron	α_2 (primary particles)	Top	>400	0.5-2.5	79-95	2-12	3-15
	β (secondary particles)	Bottom	>400				
Pre-β	α_2	Throughout tube	>400	2-10	60-80	10-20	10-20
			20-400	10-13	50-70	10-25	15-25

glycerides. These technics, collated in Table 1, will be considered one by one.

Ultracentrifugation

The preparative ultracentrifuge separates lipoproteins according to their flotation characteristics, which depend upon size and density. It can be used to separate the main chylomicron (exogenous) glyceride mass from the endogenous particles. Such separations are not complete, however, for the flotation rates of chylomicrons and endogenous particles overlap.[6,7,103] Thus, the "chylomicron" fractions prepared by exposure of plasma to centrifugal fields of about 10^5 g. per minute may contain a variable mixture of endogenous as well as exogenous particles. After this fraction has been removed a species of very-low-density lipoproteins can next be isolated that is generally considered endogenous in origin. It is of S_f 20-400 and identical to the pre-β lipoproteins separated by paper electrophoresis.[104] By manipulation of the diet of subjects serving as lipoprotein sources — for example, fat-free diets, high in carbohydrate, which induce high concentrations of endogenous glycerides, or high-fat diets, which produce transient chylomicronemia — the preparative ultracentrifuge may be used to obtain quantities of pure or nearly pure particles of the two types.[7,78]

Precipitation

Precipitation methods are among the oldest of the technics for preparing lipoproteins and are of great usefulness in the isolation of pure lipoproteins.[29] No reliable way has yet been found to use precipitation alone for precise subfractionation of very-low-density lipoproteins and particles. Dextran sulfate, amylopectin sulfate, heparin or polyvinylpyrrolidone precipitate endogenous and exogenous particles together.[29] The combination of gradient flotation and precipitation by polyvinylpyrrolidone is used to separate chylomicrons into 2 classes, "primary" and "secondary" particles, and these exogenous particles can also be separated from endogenous particles.[6,105] This technic can be employed to distinguish endogenous and exogenous hyperlipemia and is particularly useful for preparative work since the flocculated particles can be recovered for lipid and other analyses.

Electrophoresis

It was noted quite early in the study of plasma by free electrophoresis that the turbidity in lipemic samples obscured underlying protein peaks.[106] Exogenous particles in lymph were shown to migrate with albumin whereas those in plasma moved with β globulin. When the simpler technic of electrophoresis in an inert medium of starch granules was devised, similar turbid peaks were found and the separateness of an α_2 lipoprotein (pre-β lipoproteins on paper electrophoresis) established.[107] More recently it has been demonstrated that exogenous particles in plasma may accumulate in 2 regions on starch blocks.[6,108] Exogenous particles in the α_2 zone are called "primary" because they appear in plasma early after fat ingestion. The other group of exogenous particles appear somewhat later in the β zone and are called "secondary." The primary particles tend to be superimposed upon endogenous lipoproteins and particles when the latter are present.

Similar separations can be achieved with the simpler technic of paper electrophoresis. It was early noted that with barbital buffer, chylomicrons remain at the origin, as do the larger endogenous particles, whereas the smaller particles and S_f 20-400 lipoproteins form a "pre-β" band.[104,109,110] When albumin is added to buffer to decrease adsorption to the paper sharper separations are obtained.[111,112] The chylomicrons (both "primary" and "secondary" exogenous particles) remain at the point of application; the endogenous glyceride-rich lipoproteins are concentrated in the pre-β position, with trailing of the larger endogenous particles between the origin and pre-β region.[7,112]

Nature of Pre-β Lipoproteins[78]

The pre-β lipoproteins and endogenous particles cover a wide density spectrum varying from 1.006 to 0.93. Their composition depends on their density. As the density decreases, the relative proportion of glyceride rises, and that of protein falls. The fatty acid pattern of a given subclass is not the same in all subjects; grosser differences may obtain between normal and abnormal subjects. Nevertheless, certain generalizations can be made about the composition of the usual pre-β group of lipoproteins (Table 1). Lipids make up over 85 per cent of the total weight of the complex, protein being 2 to 15 per cent. Glyceride is the major lipid class. When pre-β lipoproteins and particles are isolated under conditions that minimize lipid exchange with other lipoproteins, the glyceride fatty acid composition is that of endogenously synthesized fat, low in linoleic acid and high in palmitic and oleic acids.[6,7]

Proteins in Pre-β Complexes[78,113]

The protein moiety of pre-β lipoproteins was for a long time considered to be mainly or exclusively the B protein, identical to that in the β lipoproteins.[114] Native pre-β lipoproteins react with anti-β-lipoprotein serums and not with anti-α serums. Certain chemical findings, however, were never explained by the assumption of a single protein. The increased electrophoretic mobility of pre-β lipoproteins over that of β lipoproteins, the higher ratio of phospholipid to cholesterol and a significant content of aminoterminal aspartic acid (the same as in the A protein) in the protein residues, spurred a search for other companions of the B protein in pre-β lipoproteins. Recently, it has been shown that after careful delipidation of pre-β lipoproteins significant amounts of both α and β lipoproteins are immunologically identifiable (Fig. 2). Hydrolysis of pre-β lipoproteins catalyzed by postheparin enzymes also immediately increases the plasma content of α and β lipoproteins. In subjects fed various experimental diets the concentrations of pre-β lipoproteins vary inversely with those of α and β lipoproteins. The evidence is good that both the A and B proteins, probably as their lipoproteins, are normally present in the triglyceride-rich complexes grouped as pre-β (very-low-density) lipoproteins.

C protein.[113] Analyses of the proteins obtained in pre-β lipoproteins and particles usually reveal some aminoterminal residues (threonine and serine) differing from those in the A or B proteins. The amounts have been variable, and the source conjectural.[1] In recent years one series of studies have dealt with the isolation of a third protein, called apoprotein C. This apoprotein has been isolated as a phospholipid-protein complex along with the A and B apoproteins after lyophilization and partial delipidation of very-low-density lipoproteins with heptane. Whether the particle sources of this protein contain glycerides of endogenous or exogenous origin has not been established. The C apoprotein is characterized as a 7S protein with hydrated density of 1.09 (gm. per milliliter) and a molecular weight of about 834,000. Although it appears homogeneous in the analytical ultracentrifuge it migrates as a double band with a prealbumin position on starch-gel electrophoresis and contains both aminoterminal serine and threonine. The C apoprotein fails to react with antiserums to α and β lipoprotein. Peptide fingerprints obtained by trypsin or pepsin digestion have been considered different from those of the A and B apoproteins. The C apoprotein apparently binds phospholipid more avidly than A or B. The bid of C protein for entry into the family of fat-transporting proteins is greeted with great interest. Although its presence in glyceride-rich particles is as indubitable as its separateness from apoproteins A and B, it remains for further work to demonstrate its specificity and its physiologic role. There is not yet sufficient knowledge about it to fit C protein into any of the concepts being developed in this review, and it does not appear in any of the accompanying illustrations.

Metabolism of Endogenous Glyceride[1,3,4,115-117]

Considerable evidence points to the liver as the major site of synthesis of the endogenous triglyceride and presumably elaboration of many if not all of the circulating pre-β lipoproteins. An important source of fatty acid substrate for glyceride synthesis is the plasma free fatty acids. The flux of free fatty acids into liver, heart, skeletal muscle and other tissues is governed by their rate of release from adipose tissue. Any factor that increases lipolysis or decreases glycerol esterification in the adipose tissue causes outpouring of free fatty acids.[118] Much of this free fatty acid is removed by the liver, and the excess beyond what it can use or store is resynthesized into glycerides and resecreted as pre-β lipoproteins since the acids apparently are not returned to adipose tissue as such. A significant "overshoot" in release of free fatty acids, therefore, may temporarily elevate the plasma glyceride concentration. Potential causes of hyper-pre-β lipoproteinemia include a number of abnormal physiologic states that are accompanied by increased release of free fatty acids.

Carbohydrate induction.[119-124] Endogenous hyperlipemia perhaps first became a distinctly recognized phenomenon in 1950, when Watkin and his co-workers[119] noted that fat-free, high-carbohydrate diets increased the glyceride concentration in a group of hypertensive men. Many others have confirmed and extended these observations. The concept of carbohydrate-induced hyperlipemia is an important outgrowth of such studies. Not all carbohydrate induction is abnormal, however, for it is now clear that the response of practically all subjects to very high-carbohydrate feeding is conversion of much of the carbohydrate to fat and its eventual release into the plasma as pre-β lipoproteins. In healthy young subjects the plasma glyceride rises to a peak three to ten days after the beginning of carbohydrate feeding and usually falls slowly thereafter despite continuation of the diet. The response is widely variable; the average rise in glyceride concentration is about 200 mg. per 100 ml. above the initial level, but in a "normal" subject may be as much as 400 mg. per 100 ml.[124] The ease of induction of endogenous hyperlipemia in normal subjects is probably due to the relatively limited rate of removal of such lipid from the plasma. The previously mentioned estimates of a turnover rate of glycerides in pre-β lipoproteins of about 2 gm. per hour in the adult have been obtained in subjects with normal and somewhat elevated glyceride concentrations.[9] The capacity of removal mechanisms to adapt to higher loads has not been established. The question of whether endogenous and exogenous glycerides are removed by an identical mechanism also has not been resolved and is important to the understanding

of certain kinds of hyperlipemia.[117] In some experiments tagged glycerides in fatty acids administered in a form comparable to the pre-β lipoproteins have been found predominantly in the liver in the fasting state and in the adipose tissue in the fed state.[116] In others, the role of the liver in removing endogenous glycerides has seemed unimportant.[125]

Particles of Exogenous Glyceride (Chylomicrons)[1,5,108,117]

Definition. The term chylomicron was coined in 1920 for the visible particles that appear in lymph and blood in response to fat feeding and contain the fed triglycerides.[126] For a time all particulate lipid was given this name, but it has become useful to return to the original meaning to provide some easy way of distinguishing dietary particles.[5]

It is easier to define chylomicrons in physiologic than in operational terms (Table 1). They float too rapidly in the ultracentrifuge to be quantified by optical means, and arbitrary centrifugal forces have been adopted for their definition and isolation. Sometimes, all particles having an S_f greater than 400 have been considered chylomicrons. Endogenous particles may also have flotation rates of this order, however, and the ultracentrifuge sometimes cannot be used to separate chylomicrons from significant quantities of endogenous particles.

In free or starch-block electrophoresis chylomicrons have a wide range of mobility. Those isolated from thoracic-duct lymph migrate with albumin.[5] As already noted, in plasma they may move with α_2 globulin (primary particles) or with β globulin (secondary particles).[108] Endogenous particles likewise have α_2 mobility and cannot always be separated clearly from chylomicrons by this technic. One separation of the 3 types of particles by polyvinylpyrrolidone gradient tubes has already been described.[6]

For clinical purposes the presence of chylomicrons in plasma is most conveniently indicated by paper electrophoresis. In albumin-containing buffer chylomicrons remain as a distinct band at the origin on electrophoresis whereas all other lipoprotein species migrate to some extent (Fig. 3).[7]

Composition (Table 1). In the light or electron microscope the chylomicrons appear to be spheres that vary in diameter from about 0.1 to 5.0 μ. The upper limit may reflect aggregation during isolation, and it is possible that circulating chylomicrons are not larger than about 1 μ. Chylomicrons, like pre-β lipoproteins, are made up mainly of glyceride, with lesser amounts of phospholipid and cholesterol. The proportions depend on the average size of the particles under study. Usually less than half the cholesterol is esterified, as compared to 65 to 75 per cent of esters in other lipoproteins. Patients with severe chylomicronemia may have a low percentage of esterified cholesterol in the plasma without the usual implication of abnormal liver function.

The protein content of chylomicrons is usually between 0.5 and 2.5 per cent of their total weight. Reports of higher protein content are probably due to contamination of the isolates. There is no general agreement about the nature of this protein or whether it is an intrinsic part of the chylomicron.[5,27,127-129] It therefore does not appear in Figure 2. Even in chylomicrons washed many times by ultracentrifugation several different serum proteins, including albumin and gamma globulin, can often be identified immunologically. Amino acid or peptide analyses on such small quantities of protein are subject to errors compounded by the probability that a mixture of proteins is usually present. Aminoterminal aspartic and glutamic acid are often obtained, and the aminoterminal serine and threonine typical of apoprotein C are inconsistently present. Considering all the available evidence, the 2 proteins obtained most consistently after delipidation are the A and B proteins. These can be seen best by immunologic means, but peptide analyses have also demonstrated the A protein on one occasion.[128] It is not known how the chylomicrons are actually stabilized, and some or all of the proteins present could be adsorbed to the surface or dissolved in these complexes without serving a functional purpose. It seems clear that upon entering plasma, chylomicrons take on additional protein, for the protein content is higher than that of similar particles isolated from lymph and, as noted earlier, the electrophoretic mobility also changes.[106] We shall have occasion shortly to examine evidence that the 2 major plasma lipoproteins have more than a casual relation to the chylomicron.

Origin of chylomicrons.[5,130] In the intestinal lumen dietary glycerides are dispersed by the action of bile salts and rendered easily vulnerable to hydrolysis by lipases. The resulting products are micelles containing glycerol, free fatty acids, monoglycerides and diglycerides that are taken up by intestinal epithelial cells and there resynthesized to triglycerides. Cholesterol, phospholipid and probably protein are added, and the resulting chylomicrons are extruded from the mucosal cell into the lymphatic spaces. The precise details of chylomicron formation and movement from the base of the jejunal epithelial cell into the lymphatics are not known. Chylomicrons travel in the intestinal lymph through the regional lymph nodes and the thoracic duct into the venous blood.

Chylomicron removal.[117] Chylomicrons are rapidly removed from the plasma. When chylomicrons containing tagged glycerides are infused into human subjects they disappear with a half-time of five to fifteen minutes.[131] From the distribution of tagged glyceride fatty acids it appears that most organs of the body rapidly receive chylomicron fatty acids. The possibility that some of the glycerides are hydrolyzed very rapidly and the resulting fatty acids distributed as free fatty acids to some tissues has to be considered in the interpretation of experimental studies of glyceride removal. It appears from such studies in several species that after an overnight fast

the major site of chylomicron removal may be the liver. In animals allowed access to food most of the chylomicrons may be cleared by adipose tissue. The concept of net hepatic removal of chylomicron glycerides has recently been challenged by experiments in which rat livers were perfused with chylomicrons in the presence and absence of heparin.[132] Without heparin, which may release lipolytic enzymes into the medium, there was no net uptake or metabolism of the particles. When heparin was added to the perfusate, uptake and oxidation began immediately. Other perfusion experiments[133] have not been in agreement, however, and the question of direct participation of the liver in glyceride removal remains unsettled.

The role of lipolysis.[117,134] It is fairly certain that a hydrolytic step must initiate or accompany the removal of chylomicrons from the circulation. The evidence is inferential and of the following nature. Although the endothelium of the hepatic sinusoids may contain spaces that could admit chylomicrons, the capillary walls in other tissues appear continuous. Passage of chylomicrons through the endothelial wall by pinocytosis has not been convincingly demonstrated by the electron microscope. Furthermore, during removal of glycerides, the fatty acids appear quickly in the free fatty acids, perhaps 10 per cent of which represent fatty acids coming in with the glycerides.[135] Tagged fatty acids appearing in liver do not remain in the glycerides in which they were delivered but are rapidly reshuffled, many appearing in phospholipids. The initial hydrolysis of the glycerides entering extrahepatic tissues is believed by some to be catalyzed by lipoprotein lipase concentrated in the capillary wall.[117] Lipoprotein lipase catalyzes the hydrolysis to fatty acid and glycerol of glycerides in chylomicrons and other lipoproteins, and will also split artificial fat emulsions if they are first activated by added serum or plasma. Parenteral administration of heparin and other polyanions causes lipoprotein lipase activity to appear rapidly in plasma. The activity rapidly disappears if liver function is normal. There is evidence that postheparin lipolytic activity is due to more than 1 enzyme, including a phospholipase.[136] The intravascular lipolysis induced by heparin or similar agents is a gross exaggeration of normal chylomicron removal to the extent that the fatty acids cannot all be removed by the immediately adjacent tissues. The free fatty acid content does rise slightly during normal chylomicron clearing. How much lipoprotein lipase is present in the adipose tissue cells themselves as opposed to their capillary epithelium is unclear.[137] It has been detected in hepatic-vein blood[138,139] but never convincingly demonstrated in liver parenchymal cells. The level of lipoprotein lipase in adipose tissue bears a relation to the insulin activity. In rats made diabetic with alloxan the enzyme activity is low and restored to normal by insulin treatment.[140]

Recapitulation. Plasma glycerides are transported by 2 different systems. Dietary fat is carried from the intestine into the bloodstream as large particles (chylomicrons), consisting mainly of glycerides. Phospholipids, cholesterol and a little protein are also present and probably stabilize the particle. The presence of chylomicrons can be established, and the quantity estimated by several technics. One of the simplest and most useful is based on the lack of mobility of chylomicrons on paper electrophoresis. The plasma concentration of chylomicrons is variable and dependent upon the timing of fat ingestion. Only quantities below the limits of detection by paper electrophoresis are normally present after an overnight fast. The disposal of these particles is relatively rapid and depends upon 2 major steps. The first is hydrolysis, which possibly takes place at the capillary wall and is catalyzed by lipoprotein lipase. The second is the ability of tissues beyond the capillary wall to take up the fatty acids released. In the adipose tissue, one of the major sites of disposal, re-esterification depends upon an adequate supply of α glycerolphosphate, which in turn is derived from glucose breakdown. The fate of the other constituents of the chylomicron, which seem to include small amounts of α and β lipoproteins, is not known. It is possible that chylomicrons after removal of some of their glycerides become pre-β lipoproteins. Alpha and β lipoproteins, freed of their mantle of glycerides, may join others of their species in the plasma.

Glycerides synthesized in the liver either from carbohydrate or from re-esterification of circulating free fatty acids are transported from that organ in pre-β lipoproteins and larger endogenous particles. These represent a broad spectrum of density and size from particles large enough to be seen in the light microscope to others merging with the density limits of the soluble β lipoproteins. The endogenous particles have higher protein and cholesterol content than the chylomicrons and different properties. On paper electrophoresis, they migrate in the pre-β range with increasing trailing as particle size increases. Their concentration in plasma changes slowly but can vary widely over the period of a few days, particularly in association with marked changes in the carbohydrate content of the diet. The removal rate of glyceride in the pre-β lipoproteins seems to be slower than that of glyceride in chylomicrons. The large endogenous particles may be cleared at about the same rate as chylomicrons, however, and it has not been shown that glyceride molecules of different origin have different disposal mechanisms.

Inherited Lipoprotein Deficiency States

Several mutations in man have been discovered that have laid to rest all uncertainty that the A and B proteins in lipoproteins have separately evolved to serve different functions. They have also considerably illuminated some of the transport tasks that

each lipoprotein seems to serve and illustrated something about the genetic control of their plasma concentrations.

In 2 genetically determined disorders the normal β or α lipoproteins respectively are completely missing from the plasma. These are abeta-lipoproteinemia and familial α-lipoprotein deficiency (Tangier disease). The latter is termed "deficiency" because small amounts of α lipoproteins are present, but they appear to be abnormal judging from available immunochemical evidence. The clinical details of these 2 diseases have been extensively reviewed, and attention can be focused here on the disabilities in fat transport that accompany absence of one or the other class of lipoproteins.

Abeta-lipoproteinemia[141 143]

In 1950, 2 patients with neurologic abnormalities and "crenated" red blood cells (acanthocytes)[144] became the first of about 30 patients with a singular disorder subsequently reported from America, Britain and Europe. At first the acanthocytosis seemed the most striking finding; a very low concentration of plasma cholesterol was then noted and absence of β lipoproteins was demonstrated in 1960.[145] Other investigators confirmed that several patients had severe deficiency or absence of all the plasma lipoproteins of density less than 1.063, including the absence of any chylomicronemia after the feeding of fat. The name abeta-lipoproteinemia was then suggested[145] and has gained general acceptance on the assumption that this describes the primary inherited defect.

The disease is usually expressed in infancy by retarded growth associated with steatorrhea and abdominal distention. Malabsorption becomes less marked in late childhood and is succeeded by progressively more severe neurologic deficits, including loss of muscle strength and nystagmus and signs of degeneration of the posterolateral columns and cerebellar tracts. Pigmentary retinal degeneration and visual difficulties appear. Life expectancy is limited; at least 1 death has been associated with persistent cardiac arrythmias. Deposition of ceroid pigment in the tissues is a prominent finding. The plasma and tissue phospholipids are unusually low in essential fatty acids. A deficiency of the latter or some other dietary constituent whose intake is dependent upon fat absorption may be the basis of the changes in erythrocyte membranes and nerve-cell structures that seem to underlie the many features of the disease. Sibs can be affected, and sometimes there is parental consanguinity, but vertical transmission has never been observed. The disease appears to be the expression of a double dose of a mutant autosomal allele. In 1 set of parents the β lipoproteins have been low,[145] but at present there is no way to distinguish most of the presumed heterozygotes.

The plasma lipoprotein pattern in abeta-lipoproteinemia is illustrated in Figure 4. The evidence indicates that there is no β lipoprotein in plasma.

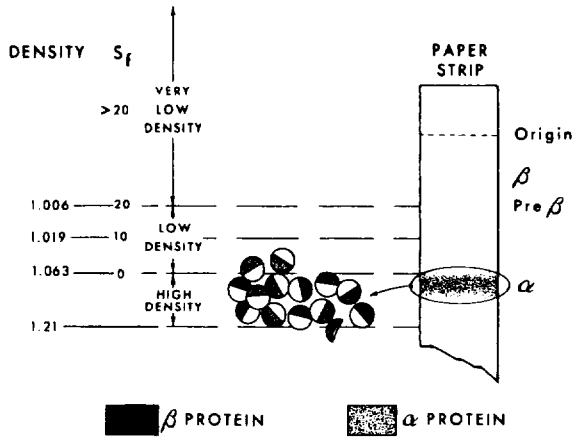

FIGURE 4. *Lipoprotein Pattern in Abeta-lipoproteinemia.*
The key clinical features are hypolipidemia and clear plasma, malabsorption, cerebellar ataxia, peripheral neuropathy, acanthocytosis, retinitis pigmentosa and autosomal recessive inheritance.

Immunochemical methods capable of detecting less than 1/10,000 of the β lipoprotein in normal plasma have revealed none in 6 patients from 4 kindreds.[79] The average plasma concentrations of cholesterol (20 to 90 mg. per 100 ml.) and of phospholipids (35 to 95 mg. per 100 ml.) are the lowest seen in any human disease. Glyceride concentrations approach the vanishing point — less than 10 to 20 mg. per 100 ml.

As shown in Figure 4 abeta-lipoproteinemia provides one of the rare instances in which α lipoproteins appear in the density region of 1.019 to 1.063 that is usually the exclusive preserve of the β lipoproteins. These α lipoproteins have a mean density that is lower than usual, probably as the result of a large lipid complement carried by the A protein. The A protein seems otherwise to be perfectly normal. The α lipoproteins are immunochemically identical to those in normal subjects, and the A protein has an amino acid composition indistinguishable from the normal.[79]

The omission of all β-lipoprotein-containing complexes (compare Figure 4 with Figure 5) is associated with the abolition of practically all glyceride transport in abeta-lipoproteinemia. The most obvious defect is a total inability to form chylomicrons.

Patients with abeta-lipoproteinemia digest glycerides, absorb the resulting monoglycerides and free fatty acids into the intestinal mucosa cells and reform them into triglycerides, but fail to release chylomicrons. Of great interest is the ability to produce in rats a similar defect in the intestine by abolishing protein synthesis with puromycin.[93,94] As patients with abeta-lipoproteinemia get older, they "learn" to absorb some fat, but must do so through another pathway such as transport of long-chain fatty acids via the portal circulation; they never have chylomicrons in peripheral blood.

When the patients are fed diets high in carbohydrates designed to induce production of endogenous

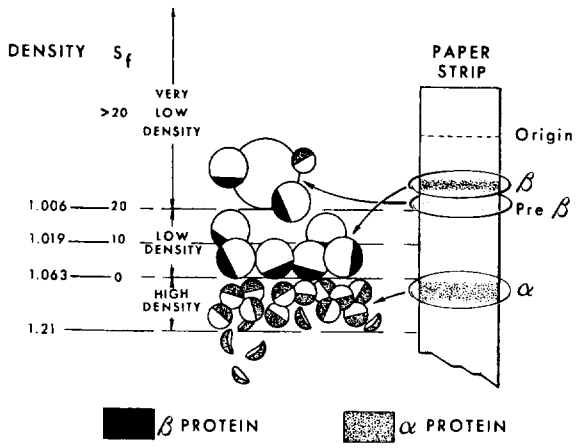

FIGURE 5. *Normal Lipoprotein Pattern in a Young Subject after an Overnight Fast.*
Pre-β Lipoprotein May Not Be Present.

glycerides and release of pre-β lipoprotein, no increase in plasma glycerides occurs and no trace of pre-β lipoproteins appears.[79] Diets that contain more than 20 gm. of carbohydrate per kilogram of body weight have been used. A liver biopsy has been obtained on 1 patient who was regularly on a relatively high-carbohydrate diet and revealed tissue containing excessive amounts of glyceride.[141] Thus, it appears that, like the intestinal mucosa, the liver in a patient with abeta-lipoproteinemia is able to produce and store glycerides but cannot transfer the lipid into the plasma.

The abnormalities found in the intestine and liver of patients with this disease, along with the nearly complete absence of glyceride in plasma, strongly invite the conclusion that the B protein has a specific function in the transport of both exogenous and endogenous glyceride from cells. This function cannot be adequately assumed by A protein.

Hypobeta-lipoproteinemia

A few patients with familial deficiency of β lipoproteins (as opposed to their absence) have been reported.[146-148] Beta lipoprotein concentrations have been 10 to 50 per cent of normal, with comparable decreases in plasma cholesterol, phospholipids, glycerides and probably in essential fatty acids. Some patients have had abnormally fragile red cells or acanthocytes and progressive neuromuscular difficulties developing in adulthood. In at least 1 of the reported families, the data indicate that the defect could be transmitted as an autosomal dominant; the mutations are probably different from that producing abeta-lipoproteinemia.

Fat absorption and chylomicron formation are not abolished in hypobeta-lipoproteinemia. Presumably, sufficient β lipoprotein is formed to meet these requirements, but it is likely that these patients are operating at minimal levels of B protein and its lipoprotein. For example, in 1 patient with severe hypobeta-lipoproteinemia and acanthocytosis neurologic symptoms developed only after the fourth pregnancy. Presumably, the hyperglyceridemia of pregnancy creates greater demand for glyceride transport and therefore for B protein.

Familial hypobeta-lipoproteinemia must be differentiated from that secondary to acute infections, other severe debilitating illnesses or malabsorption due to different gastrointestinal lesions. Undoubtedly as population surveys increase more patients will be found with similar decreases in β-lipoprotein production or increased catabolism. A spectrum of inherited states with different degrees of β-lipoprotein deficiency may eventually be uncovered.

Familial Alpha-Lipoprotein Deficiency (Tangier Disease)[55]

The analogue of abeta-lipoproteinemia was discovered in 1960 and called Tangier disease after the first 2 examples, in siblings five and six-years-old, from Tangier Island in Chesapeake Bay. Since that time the authors have had the opportunity to study 6 more examples, in 3 pairs of siblings from different families unrelated to the Tangier population.

In Tangier disease the plasma concentrations of cholesterol average 70 mg. per 100 ml. (with a range of 50 to 130 mg.), and that of phospholipids about 100 mg. (range of 70 to 140 mg.), both similar to those found in abeta-lipoproteinemia. The glyceride concentrations tend to be modestly elevated in the postabsorptive state (range of 120 to 280 mg.). The dramatic abnormality that uniquely characterizes the disease is the great size and peculiar orange color of the tonsils. Even small tags remaining after tonsillectomy have the telltale appearance. These changes are due to a gross deposition of cholesterol esters in reticuloendothelial tissues, sometimes also associated with enlargement of liver, spleen and lymph nodes. All the known clinical manifestations of the disorder appear to be secondary to the deposition of lipid, which is widespread and can also be found in cutaneous lesions and the cornea, blood vessels and reticuloendothelial cells in the rectal mucosa. One adult patient had pancytopenia corrected by removal of an enlarged spleen. His affected sibling died at the age of forty-eight years with a probable myocardial infarction.

Alpha-lipoprotein deficiency seems certain to be the primary inheritable defect, and extensive study of the pedigrees has revealed the expression in a clear genetic mode. The presence of a single abnormal autosomal gene results in abnormally low plasma concentrations of α lipoprotein (in terms of lipoprotein cholesterol, below 32 mg. per 100 ml. in males and 35 mg. per 100 ml. in females) but no significant tissue lipid deposition.[149] The homozygous abnormal genotype is expressed by near absence of all plasma high-density or α lipoproteins and tissue accumulation of cholesterol esters.

The plasma of the homozygote usually appears to be completely devoid of α lipoproteins after paper electrophoresis or ordinary immunophoresis. With appropriate antiserums and concentration of the plasma, however, a small amount of α lipoprotein

can be detected. This lipoprotein can be isolated between the densities of D 1.063 and 1.21 in the ultracentrifuge and concentrated in a manner similar to that of normal α lipoprotein. It contains cholesterol and phospholipid and has an α_1 mobility like that of α lipoprotein. It also reacts with anti-α-lipoprotein serum, and when used as an antigen, provokes antibodies to itself as well as normal α lipoproteins. Despite these similarities to native α lipoprotein, however, further immunochemical studies reveal that the Tangier α-lipoprotein, in both its lipidrich and its delipidated form, is not antigenically identical to normal α lipoprotein.[150] Thus, it has been given the separate designation, Tangier α lipoprotein; it is present in the plasma of patients with Tangier disease in about one twelfth of the concentration of α lipoprotein in normal subjects (about 30 mg. vs. 360 mg. per 100 ml.) and is the only form of α lipoprotein that can be identified by immunochemical technics. The heterozygous relatives of the patients have both Tangier α lipoprotein α_1 and α lipoprotein.

The β-lipoproteins in Tangier disease appear to be normal on immunophoresis. Their phospholipid content is high, however, and some float at the abnormally low density of 1.006.

The lipoprotein pattern in postabsorptive plasma from patients with Tangier disease (Fig. 6) is unique. The paper electrophoretogram alone immediately permits a presumptive diagnosis that needs only to be confirmed by immunochemical analyses.

In contrast to abeta-lipoproteinemia the patients with Tangier disease absorb fat normally from the intestine and make chylomicrons that behave physically like the normal ones. Their ability to release endogenous glyceride into the circulation in response to a dietary carbohydrate load is also unimpaired.[78] This endogenous hyperlipemia is associated, however, with peculiar triglyceride-laden lipoproteins of density less than 1.006 that appear as a broadened β band on electrophoresis (Fig. 6). This phenomenon provides a convincing demonstration that the normal pre-β mobility of the very-low-density lipoproteins depends upon the presence of α lipoproteins in the complex.[78]

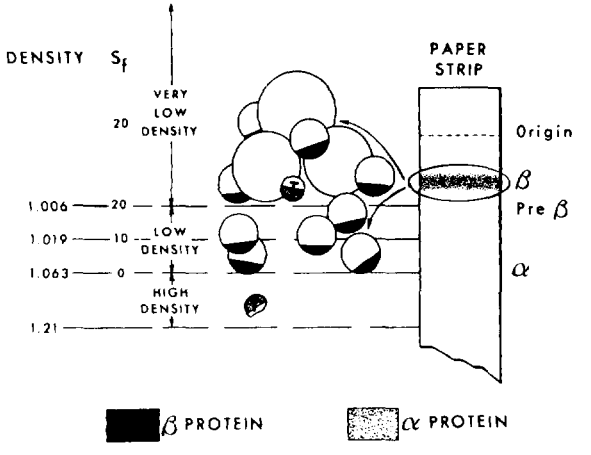

FIGURE 6. *Lipoprotein Pattern in Tangier Disease (α-Lipoprotein Deficiency).*

The small quantity of α lipoprotein is marked with a "T" to indicate that the molecule is abnormal. Clinical features are low cholesterol, normal or elevated triglycerides, enlarged tonsils and hepatosplenomegaly due to cholesterol-ester storage and autosomal recessive inheritance.

As best as can be determined from immunochemical studies the reticuloendothelial tissues in patients with Tangier disease do not seem to contain α lipoproteins in unusual quantities, and there is no evidence that the cholesterol-ester infiltration arises because cells have engorged α lipoproteins. A single study has revealed no increase in cholesterol synthesis in the Tangier tonsil. It seems more likely that α lipoprotein enhances the stability of the circulating particles containing glyceride and other lipids. When the lipoprotein is missing, the unstable particles may be more susceptible to removal by phagocytic cells. The cholesterol esters may represent the steadily increasing residue of such an uptake.

Recapitulation

Information derived from the observation of patients with lipoprotein deficiency combined with other information permits several hypotheses concerning the participation of 2 particular proteins in fat transport. These are briefly summarized as follows. The liver and intestine, and perhaps other tissues, have the capacity to synthesize proteins with a special affinity for lipids. In extracellular fluid these proteins appear in association with characteristic and different amounts of phospholipids and sterols to form 2 independent kinds of lipoproteins. These α and β lipoproteins not only provide a means for keeping their constituent lipids in solution but also assist in the stabilization of more lipids when they must be transported in plasma. The glycerides, one of the major forms in which fats destined for ultimate caloric use are carried, seem to depend upon lipoproteins for their movement out of cells and delivery to sites of removal. The B protein, backbone of β lipoprotein, appears to have a special function in making it possible for intestinal

and liver cells to deliver glyceride into the extracellular fluids. How and at which cellular sites or surfaces it acts in this capacity is not known. In man, at least, the A protein, which forms the α lipoproteins, cannot perform this function. Its absence is reflected in somewhat poorer removal of glyceride from plasma and tremendous and progressive cholesterol accumulation in tissues.

Many control mechanisms must operate to maintain plasma lipoprotein concentrations. Ultimately, these affect the rates of synthesis and catabolism of the lipoprotein proteins, but they probably are more directly responsive to changing requirements for lipid transport. The constantly shifting demand for glyceride transport requires a quick response. Other requirements, such as the need for stepped up movement of cholesterol when this lipid is ingested or synthesized in increased amounts, perhaps allow more time for adaptation. The states of metabolism of carbohydrate and protein also influence lipoprotein concentrations, and the total number of genetic and environmental factors at play is obviously large. If even the least subtle of all the possible controls on lipoprotein metabolism were completely clarified it would be much easier to discuss the clinical problem of hyperlipoproteinemia. As it is we must take up that topic in the next section, employing terms more descriptive and less mechanistic than one would desire.

HYPERLIPOPROTEINEMIA

Definitions

Up to this point we have concentrated on laying the support for 2 generalizations. The first is that, with the exception of free fatty acid concentrations, which have no lipoprotein equivalents, all abnormalities in plasma lipid concentrations or *dyslipidemia* can be translated into *dyslipoproteinemia*. The second is that the shift of emphasis to lipoproteins offers distinct advantages in the recognition and management of such disorders. We have already discussed the relatively few cases in which *hypolipoproteinemia* is a clinical problem, and the remainder of the review will be devoted to *hyperlipoproteinemia*.

Hyperlipoproteinemia (hyperlipidemia) falls into 2 major subdivisions from the standpoint of differential diagnosis on the basis of etiology. The *secondary* kind is an expression of altered metabolism due to some other recognizable disease, such as the nephrotic syndrome or hypothyroidism. One deals with the disease and ignores the symptom; hyperlipoproteinemia will go away if the underlying disease is successfully managed. *Primary* hyperlipoproteinemia includes all that is left. It is either *familial* or *sporadic*. The term *heritable* is less commonly used than familial, but it is more accurate if there is good evidence of genotypic variation. Familial hyperlipoproteinemia obviously need not be inheritable if it is due, for example, to patterns of excess in diet or alcohol intake that have been acquired by close relatives.

In a desire to use more specific terminology certain durable nouns from the "lipid era" should not be discarded. *Hypercholesterolemia* and *hyperglyceridemia* convey exact meaning, as does *hyperlipemia* (hyperglyceridemia severe enough to cause lactescence in plasma). *Exogenous hyperlipemia* is synonymous with *fat-induced hyperlipemia*. *Endogenous hyperlipemia* is sometimes *carbohydrate induced*, but these terms are not necessarily synonymous. Two venerable modifiers, *essential* and *idiopathic*, must be laid to rest. Their use no longer defines specific diseases and merely conceals heterogeneity. A diagnosis of "essential hyperlipemia," for example, should no longer be acceptable to either physician or patient.

Sorting out the Abnormals

There is no single test or maneuver that infallibly separates all those who have hyperlipoproteinemia from those who do not. The majority of laboratories still employ a combination of chemical measurements of plasma lipid concentrations for this purpose.

Lipid Determinations

The simplest screening method is a measurement of the total lipid content of plasma. Useful methods are available.[151] Some are relative, such as those depending on turbidity[152]; others, like the measurement of total fatty acids,[153,154] are more specific but also more complicated. All need careful standardization. Sometimes, laboratories report "total lipid" concentrations that are less than the sum of the concentrations of several lipid classes reported concomitantly. Measurement of total lipids alone never provides a specific diagnosis, and occasionally abnormal but reciprocal changes in cholesterol and glyceride concentrations can occur without throwing total lipid concentrations into a clearly abnormal range.

Combination of lipid analyses. Methods for determining cholesterol concentrations are widely available[155] and have even been successfully automated.[156] One should be included in any simple scheme for screening for hyperlipoproteinemia. It should be used in combination with 1 of 3 other tests or maneuvers: inspection of the serum for turbidity (a gross test for hyperglyceridemia); or the determination of either total lipids or glycerides. The best combination is measurement of cholesterol and of glyceride concentrations.[157,158] If both are *clearly* within normal limits, hyperlipoproteinemia is ruled out with a degree of precision that is quite adequate for current use.

Phospholipids. The addition of a determination of the total phospholipid concentration to plasma lipid analyses is not difficult. When the ratio of plasma cholesterol to phospholipids is high it indicates a relative preponderance of β lipoproteins. It is relatively low in the great excesses of α lipoprotein that may accompany obstructive liver disease. Phospho-

lipid determinations do not offer unique information about most other types of hyperlipoproteinemia, however, and they do not supplement cholesterol and glyceride determinations in such a way as to eliminate the added value of lipoprotein determinations. Plasma phospholipid concentrations[159] have therefore not been included in this review.

Lipoprotein Patterns

The 4 major groups of lipoproteins offer more variables than lipid determinations do. The patterns that these form can be used to diagnose several specific lipoprotein deficiency states already discussed and to segregate at least 5 different syndromes or groups of diseases associated with hyperlipoproteinemia (Table 2). Of all the methods for obtaining lipoprotein patterns only 2 kinds have the necessary range to achieve this segregation. The first are those based on flotation and use either the preparative ultracentrifuge alone or in combination with the analytical model. The latter comes closest to the ideal of defining hyperlipoproteinemia by a single operation or, more accurately, a series of such operations.[64] As now adapted the analytical ultracentrifuge has the ability to draw a continuous plot of the concentrations of lipoproteins in flotation (S_f) classes differing by very-small-density increments.[39] Such instrumentation is not generally available.

The preparative ultracentrifuge can also be used to obtain quantitative lipoprotein patterns.[34,160,161] To do so in a single plasma sample requires 3 or 4 serial runs and subsequent lipid determinations. The premium for adaptation of either the analytical or the preparative ultracentrifuge to the study of many patients is thus high in terms of both instrument cost and operational time.

Electrophoresis is less quantitative but much more convenient and economical. It is adaptable to the screening of large numbers of subjects at relatively low cost. Visual inspection of properly stained strips permits the immediate recognition of most normal patterns and certain abnormal ones of specific types. The definition of borderline abnormalities and the resolution of different types is achieved by ancillary determinations. Four additional steps have been adopted by us for sequential employment as they may be required to interpret the paper electrophoretogram. These include a determination of plasma cholesterol and glycerides, the precipitation of all lower-density lipoproteins and, occasionally, a single run in the preparative ultracentrifuge. The last is required to determine the quantity of β lipoproteins and whether the lipoproteins of β mobility have normal density.

The features of the electrophoretogram along with the further steps to its interpretation are summarized in Figure 7. The successful definition of lipoprotein patterns by such a systematic approach

TABLE 2. *Types of Hyperlipoproteinemia as Defined by Various Indexes to Plasma Lipoprotein Concentrations.**

Type	Appearance	Plasma Lipids		Paper Electrophoretic Bands	Analytical Ultracentrifuge	Preparative Ultracentrifuge	Particles Separated by Starch Electrophoresis or Polyvinylpyrrolidone Density Gradients	Precipitation with High-Molecular-Weight Polymers	Immunoprecipitation (Antiserum to β Lipoproteins)
		Cholesterol	Triglyceride						
I	Milky	↑±	↑ ↑ ↑	Chylomicrons present; all other lipoproteins ↓.	S_f 100-400 ↑	Bulk of plasma glyceride captured with centrifugation of 10^5 g/min.	Primary & secondary particles present in increased concentrations	Massive	Variable
II	Clear	↑ ↑ ↑	Normal or ↑	β ↑ ↑ ↑ Pre-β ↑±	S_f 0-12 ↑ ↑ ↑ 20-100 ↑±	Low-density lipoproteins 1.006-1.063 ↑ ↑ ↑; >1.006 ↑±.	Few, if any, particles present	Heavy	Heavy
III	Turbid	↑ ↑	↑	"Broad β" present; pre-β ↑± (requires ultracentrifuge to show beta lipoproteins of density <1.006).	S_f 0-12 ↓; 12-100 ↑ ↑ (? typical subclass pattern).	Low-density lipoproteins 1.006-1.063 ↓; <1.006 ↑ (diagnostic only if combined with electrophoresis to show beta lipoproteins of <1.006 in density).	Not known	Heavy	Heavy
IV	Turbid	↑	↑ ↑	Pre-β ↑ ↑ ↑	S_f 0-20 normal or ↓; 20-400 ↑ ↑ ↑.	Very-low-density lipoproteins of density <1.006 ↑	Endogenous ("hyperlipemic") particles present	Heavy	Variable
V	Turbid or milky	↑	↑ ↑	Chylomicron band present; pre-β ↑ ↑	S_f 20-400 ↑ (? typical subclass pattern)	Very-low-density lipoproteins of density <1.006 ↑	Primary, secondary, & endogenous particles present	Heavy	Variable

* ↑, increased; ↓, decreased.

depends very much upon the standardization of certain of these procedures. The current methods in use in large-scale studies of hyperlipoproteinemia at the Clinical Center have been employed in nearly 2000 plasma samples. A full description of the analytical sequence is in preparation. It will be summarized here to assure easier understanding of the interpretations that are to follow.

The plasma sample. Proper interpretation of lipoprotein patterns requires knowledge of when the patient took his last meal and whether his diet has been out of the ordinary. The following conditions have been maintained in the studies seeking definition of abnormal phenotypes by lipoprotein patterns: for 7 to 14 days before sampling the subject should take a diet sufficient to maintain stable body weight and containing foods in proportions normal for the population. For Americans this means about 40 per cent of calories as mixed fats, 50 per cent in carbohydrates and 10 to 15 per cent as proteins. No food should be taken for 12 to 14 hours before the sample is collected. Blood is mixed with ethylenediaminetetra-acetic acid (EDTA), 1 mg. per milliliter, and immediately placed in ice. The cells are removed by means of a refrigerated centrifuge whenever this is possible, and thereafter stored at 2 to 4°C. *Freezing irreversibly alters the lipoprotein pattern.* Upon storage the lipoprotein pattern slowly begins to deteriorate, the first changes being decreased sharpness of the lipoprotein bands. This deterioration is retarded by EDTA and is temperature dependent. Storage or shipment at room temperature is to be avoided. Under ideal conditions samples can be stored for several months if absolutely necessary.

Electrophoresis. Paper electrophoresis is performed at room temperature with the use of the Durrum hanging-strip method in barbital buffer of ionic strength *0.1, pH 8.6* and containing 0.001-M EDTA and 1 per cent albumin.[111] Either human or bovine albumin may be used. Electrophoresis is carried out over 16 hours at a constant voltage (120 V) with a current of 0.75 to 1.0 ma per strip. Whatman No. 1 paper yields the sharpest lipoprotein bands with the least degree of background staining. The optimal lipoprotein separations require equilibration of the strips for 3 to 4 hours in the closed cell before sample application, maintenance of the same level of buffer in both parts of the cell and periodic check of the pH of the buffer. Aging of the buffer is associated with a fall in pH below 8.2, and with daily use, the buffer must be changed every 1 or 2 months. We have the impression that the vertical (hanging-strip) method yields better lipoprotein separations than horizontal electrophoresis.

After electrophoresis the strips are dried in an oven at 95°C. for 20 minutes and stained by immersion in a supersaturated alcoholic solution of Oil-red-O (Allied Chemical Corporation) for 4 to 6 hours at 40°C. The samples are then rinsed with water and dried. The dye is made up by addition of 1.5 liters of ethyl alcohol, 1.0 liter of water, and 1 gm. of Oil-red-O, to a round-bottom flask fitted with a reflex condenser. The flask is heated with a heating mantle until the mixture comes to a boil and is then allowed to cool until the solution can be conveniently placed *without* filtering in a staining vessel kept in an oven at 37 to 40°C. The intensity of the staining of the strips is a function of the age and degree of saturation of the dye, and control strips must be run regularly for comparisons.

A series of related determinations are required for clarification of certain lipoprotein patterns (Fig. 7). It includes the quantification of α, β and pre-β lipoproteins in terms of their cholesterol content and establishment of whether all the lipoproteins of β mobility on paper are of the normal density (more than 1.006).

Ultracentrifugation. The lipoproteins and particles of density over 1.006 are isolated from 5-ml. aliquots of plasma by a standard technic employing ultracentrifugation of plasma at its own density for 16 hours at 100,000 × g.[5] The infranatant of density greater than 1.006, which contains the normal α and β lipoproteins, is returned to a volume of 5 ml. by the addition of 0.15-M saline solution.

Precipitation. The particles and all the lipoproteins containing β lipoprotein are separated from a 3-ml. aliquot of plasma by the addition of 0.15 ml. of 1.0-M manganese chloride and 6 mg. of sodium heparin.[42] A precipitate is allowed to develop over 15 minutes at 4°C. and is removed by ordinary centrifugation for 15 minutes at 4°C. The supernatant contains only α lipoproteins; its cholesterol content is equivalent to that in the fraction of density over 1.063 isolated by the ultracentrifuge (HDL).[78]

Quantification. The cholesterol content of the original plasma sample, the fraction of greater than 1.006 density and the supernatant of the heparin-manganese precipitation are determined. The concentrations of the several groups of lipoproteins as obtained directly or by difference are expressed in terms of cholesterol (in milligrams per 100 ml.). The plasma cholesterol less the fraction greater than 1.006 in density gives the concentration of pre-β lipoproteins and chylomicrons; the fraction of density greater than 1.006 less the α lipoproteins gives the concentration of β lipoproteins; the α lipoproteins represent the supernatant of the precipitation step. The entire set of procedures can be performed on all but very lipemic serums, in which the heparin and manganese may not precipitate the largest particles.

Electrophoresis. The fractions of density greater and less than 1.006 separated in the ultracentrifuge are also subjected to paper electrophoresis, and the mobility of their lipoprotein bands compared with each other and that of the lipoproteins in the whole plasma.

The cholesterol concentrations shown in Table 3 were measured by both the Abell modification[155] and the Autoanalyzer technic.[156] There was no significant difference between results obtained with the 2 methods. Triglycerides were determined by 3 technics.[157,158,162] There were no significant differences among results obtained by these 3 methods.

Quantification from paper strips. Many workers have shown that the α-lipoprotein and β-lipoprotein bands can be quantified from paper electrophoretic strips, either directly or after elution.[30,32] This has not been demonstrated for the pre-β and chylomicron bands. We have preferred to use the strips only for qualitative patterns. With experience one can estimate from the appearance of the strips the plasma cholesterol and glyceride concentrations to within 10 to 15 per cent of the chemical determinations. For purposes of comparison each day's electrophoresis run always includes a sample from a control subject whose lipoprotein concentrations are well known.

Setting Normal Limits

The interpretation of the lipoprotein pattern depends upon standards for normal concentrations of the lipoproteins. The setting of "limits of normal" for biologic quantities is often arbitrary. What is "usual" for one population may not be for another and is not necessarily healthy for either. Multiple modes of distribution for the concentration of plasma lipids or lipoproteins have rarely been demonstrated, and one is usually forced to rely on fiducial limits that assume a normal distribution.[163] The standards differ with age and sometimes with sex.

A set of cut-off points that help to define the usual limits of plasma concentration of the variables needed to interpret lipoprotein patterns is presented in Table 3. These are approximations, and for practical purposes, some of the limits have been combined for the 2 sexes even though modest but significant differences actually exist. The sex differ-

TABLE 3. *Plasma Lipid and Lipoprotein Concentrations in Normal Subjects.**

AGE	SEX	TOTAL CHOLESTEROL†	TRIGLYCERIDE†	PRE-BETA CHOLESTEROL†‡	BETA CHOLESTEROL†‡	ALPHA CHOLESTEROL†		NO. OF SUBJECTS
yr.		*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*		
0-19	M	172 ± 34	61 ± 34	9 ± 7	108 ± 33	49 ± 11		43
	F	179 ± 33	73 ± 34	11 ± 8	108 ± 10	53 ± 12		38
20-29	M	183 ± 37	73 ± 32	11 ± 8	111 ± 30	53 ± 11		41
	F	179 ± 35	62 ± 29	12 ± 10	115 ± 31	52 ± 9		37
30-39	M	210 ± 33	78 ± 39	21 ± 13	143 ± 27	48 ± 11		50
	F	204 ± 37	67 ± 48	14 ± 10	119 ± 31	58 ± 13		32
40-49	M	230 ± 55	90 ± 41	21 ± 9	128 ± 28	49 ± 10		67
	F	217 ± 35	80 ± 42	14 ± 9	130 ± 24	62 ± 14		44
50-59	M	240 ± 48	104 ± 45	29 ± 8	152 ± 22	47 ± 15		28
	F	251 ± 49	83 ± 46	23 ± 8	147 ± 36	59 ± 15		41
Suggested "normal limits"§:						MALES	FEMALES	
0-19		120-230	10-140	5-25	50-170	30-65	30-70	
20-29		120-240	10-140	5-25	60-170	35-70	35-75	
30-39		140-270	10-150	5-35	70-190	30-65	35-80	
40-49		150-310	10-160	5-35	80-190	30-65	40-85	
50-59		160-330	10-190	10-40	80-210	30-65	35-85	

*Population sample is derived from subjects with no evidence of metabolic disease or family history of hyperlipoproteinemia whose triglycerides <200 mg./100 ml.; all samples obtained 12-14 hr. after evening meal.

†Mean & standard deviation.

‡Obtained on smaller no. of patients varying from 13 to 27.

§Based on 95 per cent fiducial limits calculated for small samples – all values rounded to nearest 5 mg. (it will be noted that, for practical purposes, differences between sexes have been ignored except for alpha-lipoprotein concentrations).

ence in α-lipoprotein concentrations has been maintained (Table 3) since the lower limits do have value in detecting heterozygotes for Tangier disease.[149] The upper (5 per cent) limits for all the quantities are relatively high because the samples from which they are calculated are rather small. This should bias interpretation of patterns in the direction of mislabeling as "normal" subjects some who have marginal hyperlipoproteinemia.

Age-related changes. Lipid and lipoprotein concentrations do not progress stepwise as suggested by Table 3 but as continuous and nonlinear function of age that is not necessarily identical for males and females.[64,184-186]

The lowest lipoprotein concentrations are those in cord blood. The mean cholesterol concentration is about 70 mg. per 100 ml.,[167,168] and the α lipoproteins are about half and the β lipoproteins about a third of the concentrations shown in Table 3 for the youngest age decrement.[67] At this time there are practically no pre-β lipoproteins. Within the first few hours after birth the infant is forced to call upon the fat reserves that he has accumulated mainly in the last trimester of pregnancy. The initially low concentrations of free fatty acids are doubled, and the respiratory quotient begins to fall.[169] The transport of endogenous glyceride, perhaps required mainly to take care of overshoot in release of free fatty acids, begins in this early period. The mechanisms for transporting exogenous glyceride are also activated with the first feedings. Therefore, the demands upon β and possibly α lipoproteins should increase very early. Indeed, the concentration of β lipoproteins doubles or trebles within the first week of life, and lesser but definite increases in α lipoproteins also occur.[170,171] A very slow ascent in lipoprotein and cholesterol concentrations continues until well into the third decade.[172] For practical purposes pediatricians may use the limits in Table 3 for the first two decades without any correction except for the immediate postnatal period.

In the third decade there begins a "third phase" in which concentrations of β and pre-β lipoproteins rise at a new and more perceptible rate.[164,165] These increases are probably expressions of the change in fuel economy that is taking place at this time. Physical growth is ending, and the subject becomes more sedentary; caloric excess is easier to achieve, and perhaps other environmental and humoral factors come into play.

There is no general agreement about lipoprotein concentrations after about the age of sixty. From the available data it appears that the rise is over, at least for men. One must be very careful not to overinterpret lipoprotein determinations in very old subjects, and this sometimes poses difficulties in kindreds in which a younger propositus has hyperlipoproteinemia.

Naming the Patterns

In Figure 7 the terms "Type I," "Type II" and so forth appeared without comment. These are shorthand designations that were originally used to define different phenotypes of hyperlipoproteinemia because the existing nomenclature for the familial syndromes was inadequate and frequently misleading.[159,173,174] They have proved to be of such value for ready communication both in the laboratory and in the clinic that they may be used to denote specific lipoprotein patterns whether they are associated with primary or secondary hyperlipoproteinemia. The advantage is one of convenience. The nomenclature for lipoproteins is unhandy and may be confusing when different technics are being considered. For example, the synonym "Type IV" is more convenient than either "hyperprebetalipo-

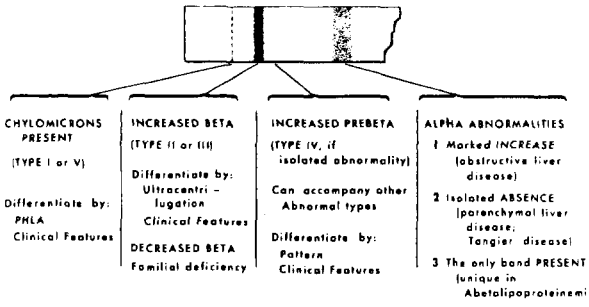

FIGURE 7. *Steps in Interpreting the Paper Electrophoretogram.*

proteinemia" or "increased very-low-density (less than 1.006) lipoproteins." Since the clinical features associated with the different patterns tend to be specific, the type designation is frequently used here for either lipoprotein pattern or syndrome. All the nomenclature for these diseases, especially the genetically determined ones, must someday be based on a description of the responsible metabolic defect. The type system is only a temporary solution.

The numbering of the lipoprotein patterns according to type has been arranged in a mnemonically convenient way. This can be seen by comparison of Figure 5, the normal lipoprotein pattern, with similar figures that follow and show the abnormal patterns. The numbering begins at the origin of the paper electrophoretic strip, with Type I referring to the presence of chylomicrons, Type II to hyperbetalipoproteinemia and so on as the bands sequentially occur on the strip.

We may now begin to examine each of 5 abnormal types of lipoprotein patterns associated with hyperlipoproteinemia. This classification is an evolving one based on continuing studies at the Clinical Center that have emphasized genetically determined disorders.[159,173-175] It must be kept in mind that the lipoprotein patterns do not necessarily reflect genetic abnormalities, nor do they imply abnormal metabolism of the lipoproteins themselves as opposed to changing demands for fat transport. A single abnormal pattern may be the expression of one of several very different diseases. Finally, it is stressed once again that the index lipoprotein pattern for purposes of classification is that obtained on a normal diet.

TYPE I HYPERLIPOPROTEINEMIA[159]

General

Definition. The Type I lipoprotein pattern, in the system underlying this review, is characterized by the presence of chylomicrons in high concentration in plasma fourteen hours or more after the last meal of a normal diet. The chylomicrons carry dietary glycerides and are particles so large that they scatter light and cause hyperlipemia that is properly called exogenous or fat induced. Of several theoretically possible reasons for this type of hyperlipoproteinemia there is at present good evidence concerning the existence of only one, a decrease in the activity of the enzyme lipoprotein lipase. Retarded removal of chylomicrons from plasma is associated with other disorders that may not involve decreased activity of this enzyme. Some of these will be taken up later under the mixed form of hyperlipemia, Type V.

Lipoprotein pattern. By the Type I pattern is meant hyperchylomicronemia in fairly pure form. Pre-β lipoproteins may be slightly increased. A *decrease* in α and β lipoproteins is the rule. Paper electrophoresis using the albumin-barbital buffer is at its most valuable in the quick and usually clear-cut demonstration of a chylomicron band (Fig. 8). Other ways to define the Type I lipoprotein pattern are listed in Table 2.

The representation of chylomicrons in so truncated a density spectrum as available in Figure 8 is schematic in the extreme. The cut-off points used in the ultracentrifuge to separate chylomicrons are arbitrary, and a precise definition by flotation is not available. A crude and eminently practical definition is that of allowing lactescent plasma to stand overnight in the icebox. A discrete cream layer on the top usually represents chylomicrons.

Plasma lipids are helpful in detecting hyperchylomicronemia, but are not definitive. If the patient is on a regular diet the glycerides will exceed the cholesterol by a ratio (milligram per milligram) of about 8:1 or higher. The proportion of free cholesterol will be high, about 50 per cent instead of the usual 30. Under the right conditions chylomicrons are precipitable by dextran sulfate or heparin along with other low-density lipoproteins. The chylomicron precipitate often does not sediment, however, and routine precipitation technics (Table 2) yield quite variable results in severe chylomicronemia. They also cannot discriminate between exogenous and endogenous hyperlipemia.

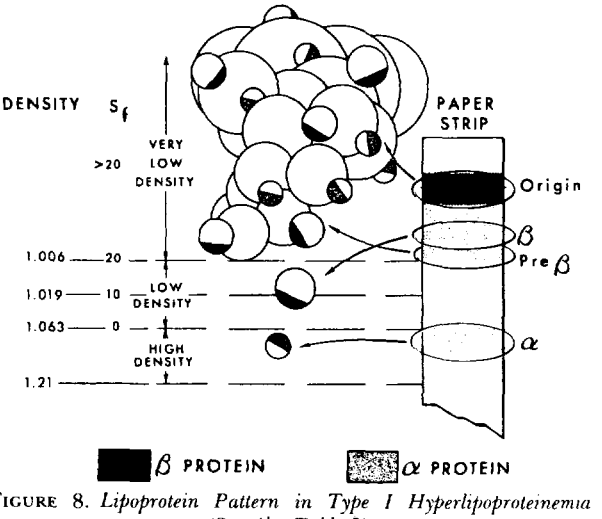

FIGURE 8. *Lipoprotein Pattern in Type I Hyperlipoproteinemia (See Also Table 3).*

The key clinical features of the familial syndrome are early expression, bouts of abdominal pain and other accompaniments of severe hyperlipemia, low postheparin lipolytic activity (PHLA) and autosomal recessive transmission.

It will be noted in Figure 8 how scanty are the amounts of α and β lipoproteins in severe hyperchylomicronemia. This is also seen with the excesses of pre-β lipoproteins or chylomicrons in Types IV and V. From evidence developed earlier it appears that these smaller lipoproteins are "bound" to or otherwise associated with the triglyceride particles so that they are no longer detectable in their usual density range or electrophoretic position.

Although concentrations of free fatty acids have not yet been proved to be helpful in diagnosis they are low in Type I and do not show the normal rise on feeding of fat. This is in accord with an assumed defect in hydrolysis of glyceride at the capillary wall.

Secondary Type I

There are cases in which abnormal chylomicronemia occurs in association with other forms of hyperlipoproteinemia. These are discussed later under Types IV and V and include difficulty in removing exogenous glyceride that appears to be secondary to such problems as uncontrolled diabetes,[176] pancreatitis[177-179] and acute alcoholism.[180] Whether hyperchylomicronemia secondary to other diseases exactly mimics the Type I pattern seen in the familial lipase deficiency is uncertain.

Primary (Familial) Type I

The most severe degree of hyperchylomicronemia is seen in patients who apparently are homozygous for a rare mutant gene regulating the activity of the clearing enzyme or enzymes. All familial Type I hyperlipoproteinemia ultimately may not turn out to represent lipoprotein lipase deficiency, but this is the only biochemical defect currently recognized.

History. The history of this syndrome has been reviewed elsewhere.[159] It is somewhat more straightforward than that of the other types of familial hyperlipoproteinemia. The manifestations are so dramatic that few if any of the earliest case reports have been ignored. The example generally accepted as the first representative of the primary familial Type I syndrome was the case of a twelve-year-old boy reported in 1932 under the title "hepatosplenomegalic lipidosis" by Bürger and Grütz.[181] The first patient with a relative who also had hyperlipemia was described in 1939 as having "idiopathic familial hyperlipemia."[182] After this description appeared many other cases were reported under the title "idiopathic" or "essential hyperlipemia," but close scrutiny of the case reports suggested that only a few of these qualify as the Type I syndrome. If fairly rigid criteria are used to define the syndrome, about 35 acceptable case reports can be found.[159] Examples of primary Type I have been described by the following synonyms: idiopathic familial hyperlipemia; essential familial hyperlipemia; retention hyperlipemia; alimentary "hepatogenic" fat retention; familial lipemia; fat-induced hyperlipemia; and familial hyperchylomicronemia: Of these definitions only the last 2 seem acceptable. One defines the characteristic lipoprotein pattern, and the other the origin of the glycerides accumulating in plasma. All synonyms; including the shorthand term, Type I, can be replaced by a more specific one of *lipoprotein-lipase deficient familial hyperchylomicronemia* when it has been ascertained that the patient qualifies for such a diagnosis. For this one needs to have certain information beyond the lipoprotein pattern, including the patient's enzyme response to heparin.

Clinical manifestations. The important clinical manifestations summarized in the legend of Figure 8 are sufficiently reproducible to permit the synthesis of a "typical case history." It may begin at a pediatrician's office with the appearance of a mother whose one-month-old child looks healthy but has "bouts of colic" and an unusually prominent abdomen. Recently, some yellow papules with a reddish base may have broken out over the skin and oral mucosa. An enlarged liver and spleen are felt, and hospitalization for observation is recommended. Here, the intern sees nearly white retinal vessels (lipemia retinalis). As the blood sample emerges in the syringe it looks like "cream-of-tomato soup." The lipoprotein pattern is established, and the baby's formula is changed to one containing only skim milk or other fat-free sources of calories. Within three days the hyperlipemia has cleared dramatically. The xanthomas will shortly resolve, the liver and spleen will decrease in size, and the apparent attacks of abdominal discomfort will disappear.

Such a child is more fortunate than the patient with the same genotype whose abnormality is not detected until he is old enough to describe his abdominal discomfort in details that suggest one of a variety of acute surgical emergencies. He may undergo laparotomy before the nature of his syndrome has been appreciated. Although the majority of patients are discovered before the age of ten, some may be adults when the diagnosis is first made.[159]

Diabetes. Glucose intolerance is not a feature of Type I and the usual oral and intravenous tests are normal even when the patient has severe hyperlipemia. This is in contradistinction to other types of hyperlipemia, including some that are "fat-induced" (see Types IV and V).

Diagnosis. The diagnosis of this syndrome is usually not difficult and entails three steps: identification of the Type I lipoprotein pattern; ascertainment that the glyceride accumulation is immediately related to dietary fat intake; and measurement of plasma postheparin lipolytic activity (PHLA).

Within a few days of switching a patient with a Type I pattern from a regular fat intake to less than 5 gm. of fat per day, one will note a *rapid* decline in plasma glycerides or hyperlipemia. The lipoprotein pattern will evolve in a predictable fashion. Chylomicrons will disappear, the β-lipoprotein band will increase, as will the α, but the most striking

change will be abnormal accumulation of pre-β lipoproteins.[7,159] The glyceride concentrations rarely become completely normal on the fat-free diet because the patient is now moderately "carbohydrate induced." Presumably, the means of removing endogenous glyceride are similarly affected by the inherited abnormality, and even the normal increase in endogenous glyceride that follows the shift to a high-carbohydrate diet causes a slightly greater hyperlipemia than normal. The paper electrophoretic technic is most helpful for observation of the fascinating and instructive changes in lipoprotein patterns in Type I with different diets.

Lipoprotein lipase activity should be assessed in all such patients.[183-185] PHLA is best assayed under in vitro conditions capable of determining maximum reaction velocity. In 12 patients from 11 kindreds we have found values from 0.04 to 0.20 (in μEq. of free fatty acids per minute per milliliter of plasma). This is below the range of values (0.24 to 0.60, mean about 0.40) found in over 100 subjects with either normal or Type II-IV lipoprotein patterns. One other patient with the familial Type I syndrome has persistently had normal PHLA, although his sibling, with an identical lipoprotein pattern, had abnormally low values.[159]

Phenocopies. Low PHLA, as defined by the assay used for the Type I patients described above, has been reported in patients with untreated diabetes[176] and hypothyroidism.[186] This activity is also low in other syndromes, and the enzyme assay is not a specific determinant of genotype. One other problem plagues the definition of the familial Type I syndrome. This is the possibility that in some of the patients the disorder has "converted" to another type of hyperlipoproteinemia because of pancreatitis, diabetes or prolonged intake of an abnormal diet. One way to be fairly certain of the diagnosis is the detection of another typical example of Type I in the patient's family.

Inheritance. In 5 kindreds with primary Type I, multiple sibs had had equally severe hyperlipemia.[159] Occasionally, very mild hyperlipemia has been present in 1 parent or 1 or more siblings. The study of kindreds has been inadequate, particularly concerning the need to demonstrate that a relative with hyperlipemia does indeed have the Type I pattern and not, for example, the much more common Type IV pattern. Both parents of an affected child may have normal lipoprotein patterns although in several kindreds at least 1 has had mild hyperlipemia. The relatively small number of involved sibs supports the assumption that a double dose of an abnormal gene accounts for the severe Type I phenotype and that a single dose of the gene may produce little or no detectable abnormality.

An unusually high number of parents or sibs have PHLA in or slightly below the lowest quartile of the distribution of the enzyme in normal subjects.[159] The slightly low activity may be associated with normal postabsorptive glyceride concentrations. As illustrated by the presence of the 1 homozygous abnormal patient with normal PHLA, a more specific assay for enzyme activity is needed. Attempts have been made to measure the enzyme in human adipose tissue.[187] The activities are very low. In 1 report, levels significantly lower than normal were found in a family with fat-induced hyperlipemia.[188]

Mechanism. The few well studied examples of Type I suggest that the inheritable defect in all has been a deficiency of lipoprotein lipase activity.[159,183,189] Hydrolysis of glyceride at sites of removal is decreased. This imposes severe limitation on the rate of clearing of chylomicrons and probably that of glycerides in other particles or lipoproteins. The patients with familial Type I syndrome have almost no threshold for fat removal, as though the entire normal clearing mechanism were inoperative. This presents something of a paradox, for the evidence is far from convincing that lipoprotein lipase is present in the liver. It seems either that the liver does not have an essential role in clearing chylomicrons or that lipoprotein lipase deficiency is not the basic defect in Type I. The generalized explanation of this disorder therefore hangs upon better understanding of the physiologic mechanisms of fat clearing.

There is a need to examine carefully all new examples of Type I to be certain that lipoprotein lipase deficiency is indeed present. Among other possible mechanisms, the production of abnormal chylomicrons or presence of circulating inhibitors of lipoprotein lipase has been eliminated in some patients.[159] Defects in the re-esterification or further utilization of glyceride fatty acids that theoretically could limit the rate of clearing have not been excluded, but the persistently low levels of free fatty acids in Type I argue against such possibilities.

Management. The outlook for patients with the "pure" Type I familial syndrome is not yet predictable. The well documented cases still include a relatively young group. Most have learned they must limit their daily fat intake if they are to avoid bouts of abdominal pain. The origins of the pain are still obscure, and it can occur as well in patients with other kinds of severe hyperlipemia. Sometimes, it is accompanied by the usual chemical signs of pancreatitis. It has been speculated that this organ is compromised by fat embolization or perhaps local lipolysis, giving rise to irritatingly high free fatty acid concentrations. Proof is lacking for any mechanism. On other occasions, similar pain is not associated with any rise in serum lipase or amylase content. The pain can be restricted to a single organ such as the spleen, which may be exquisitely tender. Because pregnancy may exacerbate hyperlipemia special care must be taken with these patients during this time. Several patients have delivered normal babies.

In addition to the painful attacks, the retarded removal of dietary fat has a number of side effects.

Chylomicrons awaiting access to their usual sites of disposal seem to be prime targets for uptake by reticuloendothelial cells. Large foam cells appear in the bone marrow; eruptive xanthomas arise in the skin, and the liver and spleen enlarge. These changes are less dramatic but also urge limitation of fat intake in the hope of avoiding possible compromise of the functions of these and other tissues.

Atherosclerosis. In the 35 or so patients with the familial Type I syndrome there has so far been a lack of evidence of accelerated coronary-artery disease. This is not proof, of course, that a high concentration of glycerides in plasma in the form of chylomicrons represents no particular hazard for the vessel wall. The inference is tempting, however, and may be used at least in the argument against extreme limitation in fat intake. For in these patients, this will lead to accumulation of pre-β lipoproteins. The latter seem to have a less benign association with vascular disease.

Diet. The treatment of Type I, then, is *moderate* restriction of dietary fat. The best motivated of these patients will usually select about 20 to 25 gm. of fat per day. So far as the degree of saturation of the fatty acids in the dietary glycerides is concerned, the source of the fat makes no difference to the degree of hyperlipemia.

It has been popular in recent years to feed triglycerides containing medium-chain-length fatty acids (MCT) to patients with abnormal absorption or fat-induced hyperlipemia. This permits fat intake without chylomicron excess, since these fatty acids are taken into the body by a different mechanism, apparently through the portal system. This type of fat may induce higher concentrations of pre-β lipoproteins, and its long-term safety is uncertain.

Parenterally infused fluids and no oral intake are the best treatment of the acute abdominal symptoms that may accompany Type I. Intubation may be necessary to relieve distention and ileus.

Other "antihyperlipemic" medications thus far available have not been shown to have a place in treatment of this disease. All the patients have some rise in free fatty acids in response to heparin injections, and this drug will promote some intravascular lipolysis. The small amount of lipolysis is not enough to clear the plasma, however, and the use of heparin in Type I is without good rationale.

"Intermittent" Primary Forms of Type I

Examples of severe intermittent hyperlipemia resembling Type I may occur. We have examined the plasma of a twelve-year-old girl (a patient of Dr. Allen Crocker) whose severe, "fat-induced" hyperlipemia seemed to be intermittent even on a regular diet. She had a typical Type I pattern on one occasion and a completely normal pattern some weeks later. There was no familial hyperlipoproteinemia. Such a case is puzzling in the extreme. It can be easily calculated that if one absorbed all of a 100-gm. daily intake of fat, and the removal of this were suddenly and completely blocked, a severe hyperlipemia of the order of 3000 mg. per 100 ml. could be attained in one day. Presumably, some "toxic" factors could so interrupt the clearing process at one step or another in a transient or sporadic fashion, but there is little or no knowledge of what such factors might be. Good examples of the inheritable Type I or their equally interesting phenocopies are hard to come by. They deserve uncommon attention.

Possible lesser degrees of Type I. In our experience with paper electrophoresis even the faintest of chylomicrons is very rarely detected in apparently healthy Americans of any age after an overnight fast. In young adults the chylomicron tide has disappeared by midnight, six hours after the last meal. When the daily fat intake, spread over 3 meals, is increased to a total load that is two or three times greater than the usual American intake, chylomicrons are still briskly removed, and the lowest glyceride measurements in the daily cycle are registered just before breakfast.

Our experience that hyperchylomicronemia is extremely unusual when electrophoresis is used for screening is not necessarily at odds with other suggestions that fat tolerance may decay with age or other conditions[190] or that minor degrees of intolerance might be genetically determined.[191] The paper electrophoretic technic is relatively insensitive and may not detect very small amounts of chylomicrons. The fast for twelve to fourteen hours is also long, and some shorter sampling time after the fat feeding might provide a better discriminant. The ideal fat tolerance test has not yet been devised. At present it is not known whether there are mild degrees of inability to remove dietary fat that are genetically determined or sporadic. Only the grosser abnormalities can be reliably detected.

Type II Hyperlipoproteinemia[159,192-196]

General

Definitions. By the Type II lipoprotein pattern we mean an increase in the concentration of lipoproteins that have discrete β mobility by electrophoresis and the normal density and chemical composition of β-migrating lipoproteins. This kind of hyperbeta-lipoproteinemia must be distinguished from another in which the density of the β-migrating lipoproteins is abnormally low, giving rise to a pattern designated as Type III in this system for defining types of hyperlipoproteinemia. The bases for distinguishing the hyperbeta-lipoproteinemia of Types II and III arise from considerable clinical and genetic evidence that they are expressions of different inheritable metabolic disorders. Type II is a common pattern; it can be a resultant of diet or secondary to hypothyroidism and other diseases, and in many patients it proves to be an expression of a mutant gene or very similar genes, appearing in relatively high frequency in many populations, including the North American.

Lipoprotein pattern. The illustration of the Type II pattern in Figure 9 depicts two important features. Foremost is the discrete increase in β lipoproteins, which on the paper electrophoretic strip is visible as a sharp band taking increased amounts of lipid stain with almost malevolent intensity when one considers the clinical implications of the abnormality. This sharp band is indicative of an increase in the low-density classes of both S_f 0-12 and 12-20. Normally, the concentration of the S_f 0-12 class is about five times that of the 12-20 class, and this differential is usually maintained in the Type II pattern.[64,194,196,197]

The second feature of the Type II pattern, and one that we are anxious to clarify, is that it must not exclude a small increase in glyceride concentrations. In analyses of 100 consecutive patients with established familial Type II patterns that are in preparation for publication, the mean glyceride concentration is about 150 mg. per 100 ml., and the upper limit about 500. This is associated with a modest increase in pre-β lipoproteins or lipoproteins of S_f 20-100.[194-197]

After comparing the glyceride and β-lipoprotein concentrations associated with several hundred lipoprotein patterns, we conclude that in Type II a given increase in glycerides will be represented by about the same increase in pre-β lipoproteins. This is noteworthy, since the overwhelming concentrations of β lipoproteins in Type II might be expected to "accommodate" abnormal amounts of glyceride without the presence of a pre-β band on the electrophoretic strip. Some Type II patients have no pre-β band (or increase in S_f 20-100 lipoproteins); others may have distinct and sometimes abnormally increased amounts of pre-β lipoproteins. The current definition of Type II[174] differs from that described by us in our first publications related to phenotyping by lipoprotein patterns.[157,173] Formerly, the combination of increased β and pre-β lipoproteins was considered one kind of Type III pattern. Type III has since been more explicitly defined (as discussed below).

Determination of hyperbeta-lipoproteinemia. The age trends of plasma cholesterol concentrations in both sexes in the general population tend to parallel those of the β-lipoprotein (S_f 0-20) concentrations.[64,198] (See also Table 3.) The concentrations of S_f 20-400 (pre-β) lipoproteins also rise with age.[64,198,199] Thus, an intense β-lipoprotein band on the electrophoretogram can definitely be interpreted as abnormal when the plasma cholesterol concentration is clearly beyond the upper limit of normal (Table 2) only if the triglyceride concentration is not in excess of 150 mg. per 100 ml. Instead of a triglyceride determination the plasma must therefore be observed to be perfectly clear. When there is uncertainty the quantity of β lipoproteins must be specifically determined, either as described earlier or by some comparable technic. When the native plasma or serum is perfectly clear the various chemical precipitation technics or those depending on antibodies to β lipoproteins yield their least equivocal results and provide a fairly reliable index to Type II hyperlipoproteinemia.

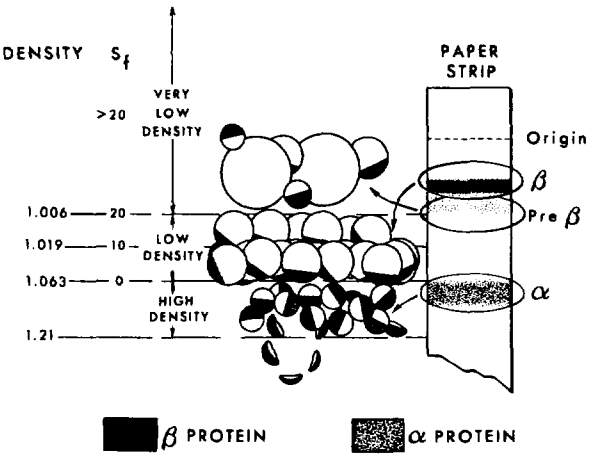

FIGURE 9. *Type II Hyperlipoproteinemia (See Also Table 3).*
The familial form is transmitted as an autosomal dominant and is usually accompanied by palpebral, tendon and tuberous xanthomas, corneal arcus and accelerated atherosclerosis. Glycerides (pre-β lipoproteins) may be modestly elevated; PHLA is normal, and glucose tolerance is usually normal.

The establishment of any dividing line between normal and abnormal β-lipoprotein concentrations is somewhat arbitrary, as already discussed. The limits provided in Table 3 are more likely to be too high than too low, at least in the context of defining the most healthy concentrations. The suggested upper limit for any age in Table 3 is 210 mg. per 100 ml. (as lipoprotein cholesterol), and in the younger age group, it is lower. The mean β-lipoprotein concentrations for the normal levels in Table 3 are very close to those in a smaller series previously reported.[161] Sets of normal values for β lipoproteins have been obtained in larger samples by means of the analytical ultracentrifuge.[64,198] The average β-lipoprotein concentrations (S_f 0-12 plus S_f 12-20) in the latter analyses, when estimated arbitrarily in terms of cholesterol content, are slightly higher than those shown in Table 3. The mean increased with age into the sixth decade in these analyses as it does in Table 3.

Major assistance in judging what are "normal" limits has been provided by kindreds with the familial Type II syndrome. Affected adult members, on a regular diet, rarely have β-lipoprotein concentrations below 250 mg. per 100 ml. Some of the young children affected and some of the adults on strict diets may fall below 200 mg. From inspection of the data being gathered in the Clinical Center studies it appears that bimodality in the distribution of β-lipoprotein concentrations within Type II families will be demonstrable. From a few Lebanese families in which several probable homozygous abnormal members appear it seems likely that 3 modes will be demonstrable when a population sample containing a sufficient number of marriages

between heterozygotes has been examined.[200] Previous attempts to show several modes of distribution of plasma cholesterol concentrations in affected American kindreds have, at the most, been only partially successful.[194,201]

Environment and the Type II pattern. The interpretation of possibly abnormal β-lipoprotein concentrations in the "gray zone" of about 175 to 225 mg. per 100 ml. (as cholesterol) is difficult. Depending on the age of the patient, the case may have to be considered primary "Type II" even though the family history is negative. Such "probable abnormal patients" are not infrequent in groups with accelerated atherosclerosis. The interpretation of their lipoprotein patterns requires special attention to their living habits. It is generally accepted from epidemiologic studies and easily demonstrable experimentally that otherwise normal subjects who eat a great deal of cholesterol[202,203] or an increased amount of saturated fats[12] generally have higher plasma cholesterol concentrations than those who eat much less of these foodstuffs. There is no consensus of whether sterol or the type of dietary glyceride is the prime determinant.[204] A β-lipoprotein concentration cannot be properly interpreted, therefore, without knowledge of the patient's diet. Although the lipoprotein level can be driven down by certain diets to much lower levels in normal subjects than in patients with the Type II pattern adequate study has not yet been made of the possibility that response to diet alone may segregate primary hyperbeta-lipoproteinemia into sporadic and inheritable forms or further subdivide patients within these main groups.

Dietary restrictions should not be imposed on a patient with a Type II pattern until the etiology has been carefully sought. The effort expended in changing a patient's food habits, and often those of his family requires that metabolic determinants other than diet be excluded and that the degree of involvement shared by the members of the family be ascertained.

Secondary Type II

Type II hyperlipoproteinemia accounts for a great deal of the "hypercholesterolemia" that the physician sees. As with other abnormal lipoprotein patterns, the most systematic approach to diagnosis and treatment first requires the elimination of certain diseases of which the hyperlipoproteinemia is a *secondary manifestation.*

Probably the most common of these is hypothyroidism. Next most common are some types or stages of obstructive hepatic disease and hypoproteinemias like the nephrotic syndrome; quite rarely one may encounter this pattern with myeloma, macroglobulinemia, idiopathic hypercalcemia and other unusual disorders. Obstructive jaundice usually provides a unique pattern that will be described separately. Others of these disorders will be discussed in the concluding section of this review under Type IV, the abnormal lipoprotein pattern with which they are more commonly associated.

Hypothyroidism. Myxedema may be accompanied by great increases in β-lipoprotein concentrations and a Type II pattern that mimics that due to mutation.[26,64,160,161] According to studies with ^{131}I-tagged lipoproteins there is a decreased rate of removal of β lipoproteins from circulation in hypothyroidism.[205,206] Carotene concentrations are high,[205] as they are in other forms of hypercholesterolemia.[207] In hypothyroidism this has been attributed to decreased catabolism of the β lipoprotein, the major carrier of carotene in plasma. Vitamin A levels can also be low.[205] It has not been proved that decreased conversion in the intestine of carotene to vitamin A does not occur in hypothyroidism, increasing the amount of carotene absorbed and transported. It is still not known how thyroid hormone regulates plasma levels of β lipoprotein. The assumption that such a regulatory defect may be independent of calorigenic activity of this hormone is the basis for frequent use of the dextroisomer of thyroxine in the treatment of hyperlipoproteinemia.[208] Hypothyroidism is sometimes associated with hyperlipemias as well. This will be discussed again in the differential diagnosis of Type IV.

The proof that a Type II pattern is caused by hypothyroidism rests on the demonstration of hormone deficiency and improvement in the pattern as replacement therapy is undertaken. Sometimes primary Type II is not discovered until the lipoprotein pattern fails to return to normal after a patient with myxedema has been made euthyroid.

Familial Type II[159,192-196]

One of the most common inheritable forms of hyperlipoproteinemia encountered in the world is Type II hyperbeta-lipoproteinemia. Perhaps it should be called a syndrome since it may be a group of similar diseases. For convenience it will be colloquially referred to here as "familial Type II." In addition to hundreds of affected American families there are in the literature easily identifiable examples in Caucasians and Negroes from many geographic areas. There is at least 1 report among Asiatics.[209] There are no clear-cut data on the gene frequency in any population, including the American, and information about its possible occurrence in some areas of the world is completely lacking. One of the major purposes of the studies that are the basis for this review has been the establishment of better criteria for ascertainment of familial Type II and the promotion of their utilization in obtaining this much needed information.

Our experience with familial Type II consists of the study, including complete lipoprotein analyses, of over 100 definitely familial examples from about 50 families and a similar number of less well studied familial cases or single patients whose lipoprotein pattern and clinical features are compatible with the syndrome. There are many other reports of single cases or whole pedigrees in the literature. A number of comprehensive studies indicate close

agreement with the present description of this disorder.[192-196,200,210]

The consideration that all familial Type II may be due to a mutation at the same genetic locus is supported by the predictability of clinical expression and genetic mode in all the patients with this lipoprotein pattern. The heterozygote seems to be nearly always recognizable by lipoprotein pattern. Depending upon unknown factors that govern the degree and speed of expression, the accompanying xanthomatosis and atheromatosis frequently seen in the syndrome will develop in many heterozygotes. They usually survive the childbearing period and may have a normal life-span.

The homozygous abnormal phenotype has increases in β lipoprotein that are nearly double those seen in the usual heterozygote, quite severe xanthomatosis and often death in childhood from cardiovascular involvement. This includes endocardial deposits of lipid, sometimes causing aortic stenosis, coronary-artery disease and occlusive disease of the peripheral vessels.

History. Familial Type II seems to have entered medical history surreptitiously, possibly as long as one hundred and thirty years ago. The date will remain uncertain because xanthomas were recognized sometime before it was realized that they were due to hyperlipidemia and long before different metabolic bases for the latter were appreciated. Palpebral xanthomas (xanthelasma) are shown without comment in Rayer's[211] atlas, published in 1835. The first descriptions of tendon and tuberous xanthomas appear under the term "vitiligoidea" in *Guy's Hospital Reports* in 1851.[212] The authors, Addison and Gull, were describing lesions associated with the hyperlipoproteinemia due to obstructive biliary disease, as were the other authors of reports of xanthomas in the English and French literature for the next fifteen to twenty years. The appearance of cutaneous xanthomas in a patient free of jaundice with a relative who had the same lesions was briefly described between 1868 and 1882.[213,214] There was no mention of plasma lipids, and the descriptions of the lesions are not convincing evidence for Type II.

Synonyms for familial Type II appeared in medical writing in roughly the following order (with the omission of certain terms for similar kinds of xanthomas): hereditary xanthomatosis; familial xanthoma; hereditary xanthoma tuberosum multiplex; general xanthelasma; xanthelasma multiplex; xanthoma tendinosum; essential familial hypercholesterolemic xanthomatosis; and, most recently, essential familial hypercholesterolemia.[192] Neither "familial hypercholesterolemia," "xanthomas tendinosum" nor "familial hyperbeta-lipoproteinemia" is entirely satisfactory, however; for the quite different pattern syndromes, Types II and III, can both be included in these categorical listings. An ideal name for Type II must await elucidation of the primary abnormality.

Diagnosis. The major features of familial Type II hyperlipoproteinemia form a triad. The first component is the lipoprotein pattern in the absence of other primary disease. The second is distribution of the Type II pattern in the family according to mendelian concepts of an autosomal dominant trait. Both these components should be present for ascertainment of what, in present-day genetic studies, can be considered the familial Type II phenotype. For practical clinical purposes the first component, when coupled with florid appearance of the third feature, – xanthomatosis, – is usually adequate for diagnosis. The 3 components of this triad deserve some further, more detailed exposition.

Hyperbeta-lipoproteinemia is the first requisite. It has already been defined in detail, and stress laid on the fact that it may be accompanied by moderate hyperglyceridemia. The small pre-β-lipoprotein band appearing in many Type II patterns does not give rise to significant hyperlipemia. Therefore, *milky serum is incompatible with uncomplicated Type II.*

The observations of Type II families have not yet established whether the plasma concentration of glyceride or pre-β lipoproteins may provide an independent variable that will segregate some families as representing a different "kind" of familial Type II syndrome. In 1 large kindred glyceride concentrations in affected and nonaffected subjects were not significantly different.[196] The "sporadic" occurrence of *modest hyperprebeta-lipoproteinemia* has the earmarks of representing a "way of life" in many Americans, and one must be very cautious in considering it a discriminant for subgrouping otherwise similar diseases.

Familial appearance of the Type II pattern is the second diagnostic feature. This includes the nearly 100 per cent expectation of a similar lipoprotein abnormality in at least 1 parent and distribution of the abnormality in siblings and other blood relatives consonant with inheritance of a "highly penetrant" trait that is recognizably expressed whenever a single abnormal allele is present. One abnormal parent will theoretically affect half his progeny. With rare exceptions, the single dose of the abnormal gene is usually expressed by abnormally high β-lipoprotein concentrations in the young child. In a single case umbilical-cord-blood cholesterol has been abnormally high,[215] but from evidence in 4 other Type II children, cord-blood cholesterol levels have been normal and have increased into the abnormal range during the first year.[216] Occasionally, expression may be delayed until the second decade.[159]

Tissue lipid deposition. Xanthomas and atheromas are very common features of Type II. The most characteristic manifestation is tendinous xanthomas, located particularly in the Achilles tendons and the extensor tendons of the hands and feet. Often, there are also tuberous xanthomas, particularly over extensor surfaces, including the elbows, knees, hands and buttocks, or periorbital xanthomas (xanthelas-

mas). In a severely affected subject soft, tuberous xanthomas may appear at many sites over the body. A corneal arcus is common,[217] assuming greater diagnostic significance the younger the age at appearance. Accelerated arterial disease, especially involving the coronary arteries, is a life-threatening feature. Some families may be relatively free of this manifestation[196]; others are seriously affected. Occlusive peripheral or cerebrovascular disease does not seem to be as common as coronary-artery disease in Type II.[194,196]

Not typical and tending strongly to militate against the diagnosis of Type II are eruptive xanthomas, planar xanthomas in the creases of palms of the hands or tuberoeruptive xanthomas. The last term refers to raised, sometimes pedunculated and often confluent intracutaneous lesions that have the reddish, inflammatory appearance of "eruptive" xanthomas, as opposed to the saccular lesions covered by normal-looking skin that are "tuberous" xanthomas in the more accepted sense. We are unaware of any patient with a Type II pattern *and* tendon xanthomas both of whose parents have been shown clearly to have no hyperlipoproteinemia. A family history consonant with such tissue lipid deposition is therefore helpful in diagnosis if the parents cannot be sampled.

It has been shown convincingly that tissue lipid deposits increase with time and are related to the degree of abnormality in the lipoprotein pattern.[193] Homozygous abnormal subjects can have xanthomas very early in childhood and may even be born with them.

Other clinical manifestations. The legend of Figure 9 summarizes the important clinical features of Type II, most of which have already been discussed. The overall incidence of "chemical diabetes" in Type II is not obviously greater than that thought to be present in the general population. A much larger study with suitable glucose tolerance tests is needed, however, to establish the true incidence and to determine whether the *hyperprebeta-lipoproteinemia* found in some Type II subjects is related to abnormal glucose tolerance. It also is not known how the presence of other abnormal genes, such as that determining diabetes, might affect the expression of Type II.

Exaggerated carbohydrate inducibility is not a common feature of familial Type II, and most of these patients can be given diets high in carbohydrate for many weeks without marked increase in plasma glyceride concentrations. Ability to clear incoming dietary glycerides does not seem to be impaired in familial Type II, and several hundred grams of fat per day leads to no chylomicronemia in the prebreakfast sample. There has been no unusual incidence of hyperuricemia in our series; a careful study of Danish patients who very probably have Type II indicates they, too, have no unusual elevation in uric acid levels.[218] Hyperuricemia is more closely associated with hyperlipoproteinemia in which hyperglyceridemia is the prominent feature.[219,220]

Therapy. It is still an assumption, but a likely one, that the hyperbeta-lipoproteinemia in familial and other forms of Type II is a major causal factor in the accelerated atherosclerotic vascular disease that accompanies this lipoprotein pattern. If some way could be found safely to decrease the hyperlipoproteinemia on a lifetime basis it would enjoy not only universal endorsement but wide application, for the number of affected patients is not small. Many regimens have been tried; none that is simple, efficacious and entirely safe has been found. Many diets or drugs or a combination of both are in experimental trial today. Some uncertainty about the results of such trials is due to failure of the therapist to identify better the syndromes with which he is dealing. For this reason it is difficult to generalize about the response of Type II to different diets or drugs at present.

Diet. Dietary management of all forms of primary Type II is justified. The degree of dietary change from the usual depends on the age of the subject, the severity of his abnormality and the nature of the previous diet. For example, a patient with a marginal Type II pattern who has neither of the other components of the triad diagnostic for the familial syndrome and who has a high intake of foods rich in cholesterol and saturated fats warrants dietary advice, for the expectation is good that he will respond. A young patient with familial Type II likewise is at such high hazard for vascular complications that dietary restrictions that do not neglect normal growth requirements are in order. In very young patients with Type II, appropriate diets can significantly lower the plasma cholesterol. For a patient over fifty years of age with a Type II pattern, there is less justification for difficult diet changes that may radically alter his way of life.

The 2 components in dietary treatment of the Type II abnormality are limited intake of cholesterol and the substitution of polyunsaturated for saturated fats. Cholesterol and saturated (usually animal) fats occur together in foods, and many therapists today are more concerned with their elimination than with the achievement of any particular ratio of polyunsaturated to saturated fats in the diet. Substitution of skim-milk for whole-milk products, severe restriction or even elimination of egg yolks and reduction in meat intake for a daily intake of about 100 mg. of cholesterol and 20 to 30 gm. of fat is one of the most effective dietary regimens.[202] Most Type II patients (provided they have normal glucose tolerance) can tolerate carbohydrate intakes of approximately 4 or 5 gm. per kilogram of body weight per day without significant rise in their glyceride concentrations. To avoid any possible carbohydrate induction, however, as well as to increase the palatability of the diets, polyunsaturated fats are often added, to make a total fat intake of 30 to 45 per cent of calories. The sources of most polyunsat-

urated fats also contain plant sterols, which have an independent cholesterol-lowering action.[12,221]

Body weight and expression of familial hyperbeta-lipoproteinemia have not been shown to bear a relation. Perhaps partly in anticipation of coronary insufficiency and other cardiac manifestations, it is reasonable to urge such patients to keep thin. A diet low in cholesterol and high in vegetable fats and maintenance of ideal weight, then, are the basic regimen for Type II.

Whatever the diet, there is always a margin of excess β lipoproteins in familial Type II patients that is persistently maintained. For example, 1 of our patients is a sixteen-year-old girl with familial Type II who, on a regular diet containing 800 mg. of cholesterol per day, has a plasma cholesterol concentration of 400 mg. per 100 ml.; 80 per cent of this is in β lipoproteins. On a restricted diet her plasma cholesterol can be dropped to about 220 mg. per 100 ml. A normal subject of the same age and on the same diet, however, will have a cholesterol concentration of about 100 mg.

Drugs.[222] Many drugs have been used in treatment of hypercholesterolemia. The 5 agents that are most commonly being tried in Type II patients are as follows, listed in descending order according to their probable effectiveness in lowering β-lipoprotein concentrations:

Cholestyramine[223] is administered by mouth in doses of 12 to 30 gm. daily. This drug binds bile acids and prevents their reabsorption. This increases the catabolism of cholesterol and may also interfere with its absorption. The dosage must be adjusted to avoid serious malabsorption of fat, and some patients find its bulk and taste quite unpleasant.

β-sitosterol,[224] in doses of 12 to 18 gm. daily by mouth, interferes with cholesterol absorption; one of the older medications still in use, it has effects that are rarely dramatic, but it has few if any important side effects.

D-thyroxine,[208] in a usual dose of 4 to 8 mg. daily by mouth, sometimes lowers cholesterol, possibly by increasing its catabolism; it is still debatable whether this action is independent of an increase in metabolic rate, which can be a serious side effect if coronary-artery disease is present.

Nicotinic acid,[225-227] in adult doses of 3 to 6 gm., daily by mouth, is capable of lowering cholesterol and glycerides by modes of action that are still to be determined but include inhibition of free fatty acid release from adipose tissue; side effects include flushing, pruritus, abdominal discomfort, increased uric acid and blood sugar and usually reversible abnormalities in liver-function tests. Its effect is greater in hyperlipemia.

Chlorophenoxyisobutyric acid,[228] usually given by mouth in doses of 2 gm. daily, is dramatically effective in some kinds of hyperglyceridemia; its mode of action is still unknown. It has thus far proved to have little toxicity and is now being used in several large-scale field trials; the available literature suggests that this drug has little effect on Type II, but experience is still meager.

Hormones. Estrogens have been shown to increase the concentration of α lipoproteins and decrease the concentration of β lipoproteins in man and lower animals.[222,229] Androgens have the reverse effect. Side effects, including gynecomastia, loss of libido and impotence, have frequently made continued therapy with high doses of estrogen impractical.[229]

In familial Type II all these agents are not likely to produce normal lipoprotein concentrations, and rebound may occur. In some patients, most tragically in severely affected children, they may have no effect. Drug therapy in Type II is therefore still experimental, even with older drugs already licensed. After appropriate attempts to classify the etiology of the Type II pattern – excluding any of the treatable causes of secondary hyperlipoproteinemia – the physician properly may elect to try one of the agents listed above, provided he closely follows the plasma lipids. There is no justification for maintaining the administration of any drug unless it clearly decreases the plasma cholesterol within a few weeks. It should also be discontinued if the cholesterol level returns to its abnormal pretreatment state or if signs of significant toxicity appear.

There is no adequate information about the response of familial Type II disease to radical procedures for upsetting cholesterol metabolism such as ileal bypass.[196,230] Since such a procedure presumably should be performed in early childhood to be maximally effective in familial Type II, it seems almost forbiddingly heroic.

The Future of Type II

Phenotyping. Just as specific therapy is urgently needed for Type II, so is wider application of means to ascertain the trait and to search for knowledge of the aberrant mechanisms. There are important questions of gene frequency and distribution, some of which could very probably be answered now if existing technics were widely used. They have direct and immediate importance in genetic counseling. The rare marriage of 2 homozygous persons portends disastrous consequences for their offspring. Counseling in such cases can be fairly definite. Marriages between heterozygotes or between a normal subject and a heterozygote make predictions more difficult, partly because phenotyping the partners is subject to more error. Physicians advising such couples about the genetic hazard to their progeny have to keep in mind the limitations of available tests for ascertainment and the fact that heterozygous offspring often have a normal life-span. Despite these uncertainties, the possibilities for identification and rational follow-up study and management of Type II families is greater than is often recognized today.

Biochemical defect. The variability of the course in different families affected with familial Type II

invites some skepticism concerning the genetic homogeneity of this syndrome. The explanation may be no more than capricious variation in location of an atheroma, but both the severity and the timing of xanthomas, as well as the concentrations of β lipoproteins, vary considerably among patients. The question will be settled ultimately when the biochemical lesions that gain expression in the Type II pattern have been specifically determined. For years the search for these has been narrowly confined to consideration of the pathways for making and disposing of cholesterol. Experimental studies of cholesterol metabolism in Type II have been summarized elsewhere[159,175,196]; they have not revealed the defect. Actually, there is no more reason for suspecting that in familial Type II the mutation affects cholesterol metabolism than that of the phospholipids or β apoprotein found in the β lipoproteins. Since the latter is unique for the lipoprotein, and the lipids not so, the metabolism of the protein remains a prime suspect. Just as the sterol and phosphatides in plasma in Type II have not been shown to be chemically different from those in normal subjects, the β protein has not been shown to be abnormal, but published data are far from complete.

The differences between the β-lipoprotein concentrations in normal subjects and in those assumed to have single and double doses of the abnormal gene for Type II suggest that the mutation or mutations responsible for the disease have affected genes governing the control of synthesis of the lipoprotein rather than its structure. The most effective approach to therapy for familial Type II may be a search for pharmacologic agents capable of suppressing the synthesis of B protein and thus replacing such missing genetic controls.

Type III Hyperlipoproteinemia

General

Definition. In the course of the phenotyping studies that form the core of this review, a lipoprotein pattern different from Type II and from Type IV, which will be discussed in the concluding section, has been found in a relatively small number of subjects. This lipoprotein anomaly has been classified as the Type III pattern. It is a feature of a clinical syndrome that was perhaps first pointed out over ten years ago[64] but is still often considered a variant of Type II. At present sufficient evidence has been gathered to indicate that the disorders associated with patterns II and III are independent. Whether the Type III pattern is the expression of a single disease or of several different metabolic disorders has not been established.

Type III hyperlipoproteinemia (Fig. 10) is an excess of lipoproteins that have β mobility but abnormally low density. It is manifested by hypercholesterolemia *and* hyperglyceridemia. A peculiar feature – and one that tips off the lipoprotein pattern if it is present – is lipid deposition in the

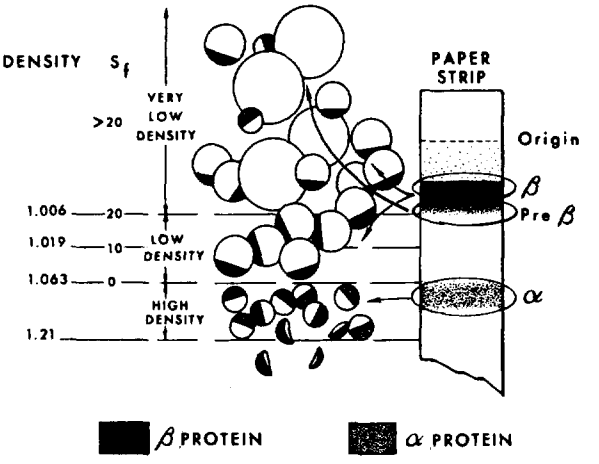

FIGURE 10. *Type III Hyperlipoproteinemia (See Also Table 3). Note that the key feature is β-migrating lipoproteins having abnormally low density (less than 1.006). In addition to arcus, palpebral and tendon xanthomas, there usually are palmar and "tuberoeruptive" xanthomas. Advanced atherosclerosis of peripheral and coronary arteries is common. PHLA is normal; glucose intolerance is the rule. When familial, it is inherited as a recessive.*

palms of the hands. The lipoprotein anomaly may be detected in close relatives and as such is probably the expression of a double dose of an uncommon abnormal gene. We have so far examined 24 Type III patients and over 100 of their relatives, and the following discussion will be based mainly on these observations. The abnormality will be referred to as the Type III syndrome or simply as "Type III."

Lipoprotein pattern. The electrophoretic abnormality in Type III is a "broad beta band." As depicted in Figure 10, there is a broad, intensely staining lipoprotein band beginning in the normal β zone but extending continuously into the pre-β region. When the plasma is subjected without adjustment of density to ultracentrifugation for sixteen hours at 100,000 g, and the supernatant and infranatant fractions again subjected to electrophoresis, most of the lipoprotein band having β mobility appears in the fraction with density less than 1.006 (Fig. 11). Normally, and in other types of hyperlipoproteinemia, all the lipoproteins of β mobility remain in the infranatant (density greater than 1.006) fraction (Fig. 11). Although adequate comparisons have not yet been made the features of the Type III pattern in the analytical ultracentrifuge can be estimated from early studies of patients who almost certainly represented the same syndrome.[64,197,231] The predominant abnormalities are lower than normal concentrations of S_f 0-12 lipoproteins, moderate increases in S_f 12-20 and marked increases in the S_f 20-100 and often the S_f 100-400 subclasses. At present a certain determination of the Type III pattern requires either the combination of electrophoresis and preparative ultracentrifugation described here or analyses in the analytical ultracentrifuge.

History. The appearance in the medical literature of the first example of Type III may never be determined. Descriptions of "mixed" hyperlipidemia

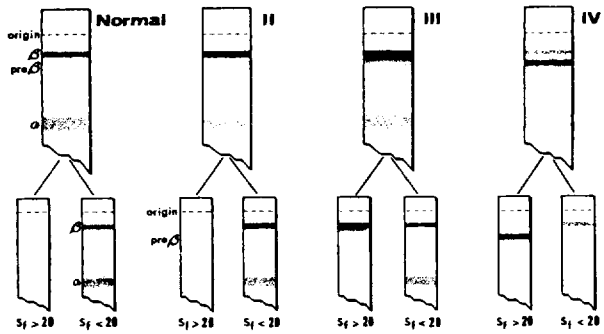

FIGURE 11. *Electrophoretic Mobility of the Lipoproteins Isolated in the Ultracentrifuge at Density 1.006.*

$S_f > 20$ represents the supernatant fraction, and $S_f < 20$ the infranate. Only in Type III do lipoproteins of β mobility appear in the 1.006-density supernate.

have been present in many reports of heterogenous patients with severe hyperlipoproteinemia and xanthomatosis.[232-236] It is inevitable that some Type III patients, with their tendency toward tendon xanthomas, xanthelasmas and crippling atherosclerosis, should have been classified as having "essential hypercholesterolemia" (Type II). Others have been singled out by their milky plasma and yellow-red eruptions on the extremities, and these cases have been consigned to "essential hyperlipemia" – a sanctuary of the undifferentiated that one hopes will soon be abandoned. One study stands out during the period from 1950 to the present, a comparison of xanthoma type and lipoprotein patterns at the Donner Laboratory.[64,197] Two groups of patients were examined, defined as having "xanthoma tendinosum" and "xanthoma tuberosum." The first group almost certainly contained examples of familial Type II; all had marked increases in S_f 0-12 lipoproteins (hyperbeta-lipoproteinemia). The other group had skin lesions on "extensor surfaces of the elbows, buttocks, extensor aspects of the knees, the hands, expecially the volar surfaces, and over the ankle malleoli, especially laterally."[197] In contrast to the patients with xanthoma "tendinosum," some of whom had lesions in childhood, all the 23 with xanthoma "tuberosum" had first noted their lesions in adulthood. Two of the latter were sibs, the only familial examples presented. In these patients the S_f 0-12 lipoproteins were lower than normal, and the S_f 12-400 classes greatly elevated.

When the classification of familial hyperlipidemia by the current system was begun at the Clinical Center, one of the objectives was the search for proof of the existence of separate phenotypes comparable to those suggested by these early studies. The present concept of Type III evolved through trial and error. In the earliest published classification by electrophoretograms[159,173] a "Type III" pattern was defined as an increase in both β and pre-β lipoproteins. It was noted that there seemed to be 2 different variations on this pattern. In 1 the 2 lipoprotein bands were intense and distinct. In the other they merged in a broad, intensely staining β band. As described above, the former pattern was eventually found to be a variant of the Type II pattern. The "broad-beta" pattern has come to define a group of patients whose familial expression and clinical features were clearly different.[174] The abnormal flotation of the β lipoproteins was noted after the quantification steps were added to the typing system. It has been observed that the β-lipoprotein anomaly is consistent and reproducible and not a technical artifact. This flotation anomaly is not a function of the absolute amounts of β lipoprotein, cholesterol or glycerides in plasma. It has never been observed in patients with Types II or IV patterns even though their plasma lipid concentrations are sometimes very similar to those seen in Type III. The important difference between the inheritance of Types II and III will be described below.

Clinical Manifestations

Plasma lipids. The plasma cholesterol concentrations in Type III vary from 200 to more than 1400 mg., and the glycerides from 175 to more than 1500 mg. per 100 ml. Extreme variability of cholesterol and glyceride concentrations is a diagnostic feature and a distinct contrast to the steady elevations in Type II. The lipoprotein levels are quite sensitive to changes in the content of the diet and the amount of total calories. If the patient is switched from a regular diet to one rich in polyunsaturated fats and containing only 100 to 200 mg. of cholesterol per day both cholesterol and glyceride concentrations may be lowered severalfold within a few weeks. The effect of negative caloric balance may be even more dramatic. Glyceride concentrations tend to rise moderately on high carbohydrate diets, but Type III is not always accompanied by carbohydrate inducibility exceeding the response of normal subjects. Carbohydrate intolerance is the rule in most patients (over 90 per cent), and the ability to handle either an oral or intravenous glucose load seems to decrease with age faster than it does in the normal state. There have been no measurements of plasma insulin levels in Type III; only 1 of 24 patients thus far has ever taken insulin or hypoglycemic agents for his mild diabetes.

The 10 patients with Type III who have been available for testing have had a small accumulation of chylomicrons after an overnight fast when they were fed more than 150 gm. of fat per day. Their hyperglyceridemia on regular diets is not exogenous, however, as witnessed by their good therapeutic response to low-cholesterol diets containing 100 gm. or more of fat. The "palmar striae" of Type III are not typical of any other type of *primary* hyperlipoproteinemia.

Xanthomas. The terms "xanthoma tendinosum" and "xanthoma tuberosum" do not accurately distinguish Types II and III. Tendon lesions occur in both, as do thickened tibial tuberosities, corneal arcus and periorbital xanthomas. The distinctive

lesions in Type III are found on the palmar surfaces of the hands. The creases and sometimes the tips of the fingers or other areas, especially where rings are worn, contain yellow-white deposits that rise a little above the surface; they are often subtle changes and are sometimes overlooked by both patient and physician. So-called planar xanthomas of similar appearance but usually on the back of the hands have long been associated with obstructive liver disease; they can be seen on models constructed from jaundiced patients over a hundred years ago.[237] Such lesions are not typical of any other type of primary hyperlipoproteinemia.

On the elbows several kinds of lesions have been observed in Type III. Soft, pedunculated growths may be present, but they tend to be confluent and smaller than tuberous xanthomas usually seen in Type II and do not have the smooth normal skin cover of the latter. The Type III lesions bear the reddish, inflammatory appearance that is the trademark of skin lesions associated with hyperglyceridemia. Sometimes, they are confluent, yellow-red papules raised just above the surface. Occasionally, when they seem to be involuting they closely resemble dabs of peanut butter spread on the skin; these last two forms may commonly be amalgamated into brownish-yellow papules having a reddish base. Similar lesions in severe diabetes have been called "xanthoma diabeticorum." For all these the name "tuberoeruptive" may be helpful in suggesting their appearance of transition between tuberous and eruptive xanthomas. The lesions wax and wane, and may even disappear without obvious change in the plasma lipoprotein pattern. Similar-appearing xanthomas may appear on the buttocks or knees. In distinct contrast to Type II, thus far only 1 patient in 24 with Type III at the Clinical Center has noted any xanthomas before the age of twenty-five.

Vascular disease. Occlusive vascular disease is a serious and frequent accompaniment of Type III. In the samples observed by us occlusive peripheral vascular disease, especially of femoral and popliteal vessels with severe claudication, has been at least as frequent as coronary atherosclerosis.

Genetics

The Type III lipoprotein pattern and other clinical features occur in families, but in contrast to Type II, the distribution of affected members is not that of a trait expressed in single dosage of an abnormal gene. The 24 patients have come from 19 kindreds. In 3 of these, 2 siblings have been involved, and in 2 others, a parent and one offspring. Fourteen have been males, and 10 females. The 104 blood relatives sampled do not include complete sets of parents and siblings from all kindreds. In 3 kindreds both parents of the propositus have been sampled. One of each set was normal. One spouse had Type III, and the other 2 Type IV patterns. It is most significant that in none of the 128 relatives and patients sampled was a Type II pattern present. Conversely, complete lipoprotein analyses of 105 patients with familial Type II and 90 of their relatives (representing 50 kindreds) failed to reveal a single instance of Type III. These include three presumably homozygous abnormal examples of Type II.

From these data it is apparent that Type III and Type II are not simply different phenotypic expressions of the same genotype. They are at least 2 different diseases. A few Type IV patterns (endogenous hyperlipemia) have been noted in the Type III relatives. As will be discussed shortly, this is a frequent pattern in Americans and may be due to factors that are not genetic. Type IV patterns appear as well in some relatives of Type II patients, and any significant differences in distribution have not developed. It is conceivable, of course, that Type III and Type IV could prove to be related, including the possible expression of the "Type III heterozygote" sometimes as a Type IV pattern. The possibility that several mutant alleles determine this pattern also remains open.

Speculation on the Mechanism

Type III, with its apparent accumulation of a subclass of very-low-density lipoproteins, requires consideration of a theory of "interconversion" of plasma lipoproteins. This is inherent in the concept developed earlier in this review (Fig. 1), which in turn evolved from even more theoretical considerations.[26,238] Briefly stated, it can be imagined that large particles containing glyceride, β and α lipoproteins are degraded in stepwise fashion. As glyceride is sequentially removed a rise and fall of lipoproteins of progressively lower S_f occurs. Metabolic block at any point in this transformation would theoretically cause accumulation of very-low-density lipoproteins of a given subclass.[26] Actually, such a sequence has never been convincingly demonstrated during chylomicron clearance[1] and a disorder of metabolism of specific subclass of pre-β lipoproteins has likewise not been proved. The tendency today is to assume that the concentration and average density of pre-β lipoproteins is a function of the amount of circulating glyceride, which in turn is determined by the state of energy metabolism at the cellular level.

It is therefore unlikely that the peculiar Type III lipoprotein anomaly is the result of a metabolic block at a discrete point in the metabolism of otherwise normal pre-β lipoproteins. The anomaly has not been observed in other types of hyperlipoproteinemia, even when the plasma concentrations of pre-β lipoproteins are being driven up or down by extreme changes in diet. For example, the glyceride levels in Type IV patients may be changed from 100 to 5000 mg. per 100 ml., and there is never lipoprotein of discrete β mobility floating at density 1.006. The Type III anomaly therefore appears more likely to be secondary to the presence of an abnormal lipoprotein.

The Type III pattern is mimicked in the "broad beta" band seen in Tangier disease (Fig. 6); in this instance β lipoproteins and glyceride form a complex that lacks the added negative charge contributed by α lipoproteins, normally giving such complexes pre-β mobility. Although the "floating beta" of Type III is suggestive of a deficient participation of α lipoprotein in formation of the glyceride-rich complexes, dietary studies have shown that these patients make pre-β lipoproteins. Because no way has yet been found to delipidate these complexes and still obtain accurate quantification of their content of α and β lipoprotein, some obvious questions cannot be answered. The regular α and β lipoproteins (in their usual density class) react with antiserums as though normal, and the content of α (as measured after heparin precipitation) is normal. One is left at the moment with the suggestion of a peculiar affinity of Type III β lipoproteins for glyceride, for "floating beta" is present in Type III patients even when diets have brought their glycerides to within normal limits. More details of these studies will be forthcoming shortly from this laboratory; they still leave Type III as a most provocative biochemical enigma.

Management

The prognosis in Type III is uncertain. Four of our patients have reached the seventh decade. The majority have evidence of vascular disease, however, and it seems reasonable to utilize such conservative means as are available to lower plasma lipid concentrations. The latter are characteristically labile and responsive to diet alone.

Diet. Weight reduction is one key to reduction of plasma lipids in Type III and is always effective if the patient is overweight. After ideal weight is reached it may be maintained by diets containing 40 to 50 per cent of calories from fats that are as high in polyunsaturated fats and as low in cholesterol as is practicable. If the diet is carefully followed, normal or nearly normal lipid levels can be maintained for many months and the skin lesions may completely disappear. It is hoped that the vascular changes may be undergoing similar beneficial involution. The "floating" β lipoprotein will persist, however, in the lipoprotein pattern.

Drugs. It is premature to comment on drug therapy for Type III since none of the agents now being investigated have been tested adequately in this syndrome. It is our opinion that drugs tending to be more effective against hyperglyceridemia than isolated hypercholesterolemia will be more effective in Type III. Agents in this category have been discussed under Type II. Perhaps the most effective and generally useful drug will prove to be chlorophenoxyisobutyric acid. Some evidence that it may lower lipid levels in Type III has been reported.[228]

Type IV Hyperlipoproteinemia

General

Definition. The Type IV lipoprotein pattern is the hallmark of endogenous hyperlipemia. It implies that glycerides synthesized in the body, usually in the liver, have been excreted into plasma at rates exceeding the capacity for removal. The appearance of a Type IV pattern often suggests that something has gone wrong with carbohydrate metabolism or caloric balance. There is evidence that it can sometimes mean inordinate emotional stress, excessive alcoholic intake or some other conflict between the patient and his environment. The development of this type of hyperlipoproteinemia is often conditioned by genotype.

The Type IV pattern (hereafter called simply "Type IV") is a valuable indicator of metabolic imbalance; it does not describe a specific disease. It is sometimes considered synonymous with "carbohydrate-induced hyperlipemia." This is probably too narrow a concept since most patients who have this pattern on a regular diet do not lose it entirely when their diet is changed so that 10 per cent of their calories come from carbohydrate, and an occasional patient does not have an *abnormal* increase in plasma glycerides when fed 80 per cent of his calories as carbohydrate. If freed of a generic connotation, the term "carbohydrate induced"[123] has great virtue, however, for it focuses immediate attention on abnormal glucose tolerance and a family history of diabetes, both associated with Type IV with extraordinary frequency.[159,239-241] At the Clinical Center, where patient selection has recently been biased toward other lipoprotein anomalies, 15 of approximately 100 kindreds with proved familial hyperlipoproteinemia have been families in which affected members had only Type IV. The pattern has been observed in several hundred plasma samples from different individuals on a regular diet. Quantitative data from a large sample representative of the entire population are not available, but Type IV may be the most common of all types of hyperlipoproteinemia.

Lipoprotein pattern. As shown in Figure 12, endogenous hyperlipemia is manifested as hyperprebetalipoproteinemia. This is associated with an increase in glycerides above normal limits as defined in Table 2 and is commonly accompanied by a rise in cholesterol, on the order of about 1 mg. for each 5-mg. increase in glyceride concentration. With the use of other analytical methods Type IV is defined by increased concentrations of S_f 20-400 and sometimes S_f 400 lipoproteins in the analytical ultracentrifuge, very-low-density lipoproteins (density less than 1.006) as separated by the preparative ultracentrifuge, lipoproteins and "endogenous" particles having α_2 mobility on starch-block electrophoresis or intermediate density by polyvinylpyrrolidone fractionation, or low-density "beta" lipoproteins as measured by most precipitation technics. Concentrations of α and β lipoproteins are usually below normal in the Type IV pattern; chylomicrons are not present.

The Type IV pattern is easy to spot by the electrophoretogram when lipemia is not extreme (glyc-

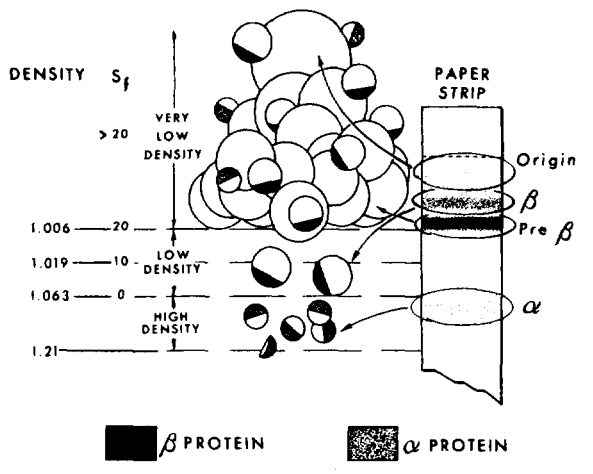

FIGURE 12. *Type IV Hyperlipoproteinemia (See Also Table 3) — Endogenous Hyperlipemia That Is Typically Carbohydrate Inducible and Accompanied by Glucose Intolerance.*

PHLA is normal, and hyperuricemia is common. There are many causes, and the pattern is often familial.

erides less than 2000 mg. per 100 ml.). In severe hyperlipemia the trail of endogenous particles from the origin is marked, and interpretation difficult. Diluting plasma onefold or twofold with saline solution may help to distinguish any boundary between pre-β lipoproteins and chylomicrons (the two together being defined as the Type V pattern). The Type I pattern is not likely to be confused with either Type IV or Type V.

The ability of paper electrophoresis to separate endogenous from exogenous glyceride appears to depend on the comparatively greater ratio of mass to charge of the dietary particles. The distinction is not absolute, and it is likely that some fed glycerides may quickly appear in pre-β lipoproteins. Patients with even the most severe endogenous hyperlipemia, however, do not have chylomicron bands in the absence of fat in the diet. Conversely, patients with Type IV who have accumulated heavy prebeta bands on high-carbohydrate diets usually have transient hyperchylomicronemia for a few days after fat is returned to the diet. As with all other types of hyperlipoproteinemia, the pattern obtained after a week or two of a normal diet must be the index for purposes of classification.

In Type IV the concentrations of β and α lipoproteins vary with the degree of hyperlipemia. As heavy concentrations of glycerides are reduced, the 2 lipoprotein bands become progressively greater. The rise and fall in β-lipoprotein concentrations is an especially prominent feature of the transition in the Type IV pattern induced by dietary changes.

History. Observations of patients with ketosis and uncontrolled diabetes who had hyperlipemia despite malnutrition probably provided some of the earliest stimulus to a concept of endogenous hyperlipemia. Other patients with lactescent serum that did not improve markedly upon withdrawal of fat from the diet have often been observed. With the development of the concept of carbohydrate induction all doubt has been removed that excess glycerides in the plasma are frequently not of dietary origin. Interest in postabsorptive increases in plasma glycerides, or what are here called pre-β lipoproteins, has also been maintained by many studies, not always in agreement, of an uneven distribution of this phenomenon in different populations. There has been special interest in whether this abnormality is unusually common in young and middle-aged adults who have ischemic heart disease.[241-245]

What are lacking almost completely in the literature are family data in clear-cut examples of Type IV. We have summarized elsewhere a few reports of what seem likely to have been Type IV patterns occurring in siblings or in succeeding generations.[159] Exploration of the pedigrees of affected patients has been distressingly meager and must be improved if the meaning of the abnormal pattern is to become clearer.

Clinical Manifestations

It is perhaps not pertinent to discuss the clinical features of what is only a chemical indicator, but patients with Type IV tend to have certain general characteristics that can be summarized as a prelude to discussion of primary and secondary forms of this hyperlipoproteinemia.

Common features of hyperlipemia. Severe Type IV from any cause may show some abnormalities already described in exogenous hyperlipemia, such as eruptive xanthomas, lipemia retinalis and foam cells in bone marrow and other reticuloendothelial tissues. The patients are also subject to the same bouts of abdominal pain — with or without chemical signs of pancreatitis. Hyperuricemia occurs in hyperlipemia,[219,220] but its frequency in Type IV has not been established.

Obesity and glucose tolerance. Secondary Type IV can occur in thin, nondiabetic subjects. So can the primary form, but in patients with the latter, excessive body weight and abnormal glucose tolerance are extremely common. In our experience the incidence of abnormal glucose tolerance in those with familial Type IV patterns exceeds 90 per cent.

Age at detection. Plasma triglyceride concentrations rise with age (Table 2). Apparently so does the incidence of Type IV. Familial occurrence has been seen in young children, as has the secondary form, but the overall frequency in children seems much less than in adults.

Atherosclerosis. All the evidence concerning the association of coronary-artery disease and pre-β lipoproteins is retrospective; some of it is derived from plasma glyceride concentrations, and some from lipoprotein analyses. Agreement is lacking, but the consensus indicates a positive correlation at least in relatively young men.[242-246] Noteworthy is the finding of a much higher concentration of pre-β lipoproteins in males who had had a myocardial infarct than in controls.[243] The possibility that many patients with infarcts are placed on very low-fat, and

hence high-carbohydrate diets may be a factor in enhancing the lipoprotein abnormalities. Our collection of about 200 kindreds with all types of familial hyperlipoproteinemia cannot yet be properly matched for age and sex for estimation of the incidence of atherosclerosis. The available experience leaves the impression that Type IV is not associated with atherosclerosis to the same extent as Types II and III. Diabetes and obesity complicate such comparisons, and only more data of the proper kind will eventually resolve this important question.

Mechanisms in Type IV

Two abnormalities may be operative in production of Type IV. A brief summary of them can be confined for simplicity to the interplay between adipose tissue and liver. The roles of both organs in normal endogenous glyceride metabolism have been discussed earlier and need not be repeated.

Excess glyceride release. The first possible abnormality is release of glycerides by the liver faster than the adipose tissue can remove them. In the steady state a new equilibrium will be established at some higher plasma level of pre-β lipoproteins. Examples in which this undoubtedly occurs include excessive flow of free fatty acids to the liver or extremely high-carbohydrate feeding. Both substrates in great excess will be turned out by the liver as glycerides that usually seek storage in the expansible reservoirs of the adipose tissue but may linger in the plasma.

To demonstrate steady or cyclical increases in flux of free fatty acids in patients is not simple; it depends in part on the determination of a plasma substance whose concentrations are notoriously labile. Increased mobilization of fatty acids has been invoked to explain clinical examples of endogenous hyperlipemia. The evidence is convincing only in diabetic ketosis but highly provocative in some examples of prolonged emotional stress.

Carbohydrate induction, on the other hand, is easy to demonstrate.[123,124,159] It may not, however, be assumed to be the defect in any patient with Type IV unless the patient's response is clearly outside the normal degree of carbohydrate inducibility – a rise in glycerides to levels as high as 300 to 400 mg. per 100 ml. after four to seven days on a diet containing 6 or 7 gm. of carbohydrate per kilogram of body weight.[124] The issue of exaggerated carbohydrate inducibility may not be solved by the use of carbohydrate loads that far exceed those the patient is usually eating when he has hyperlipemia. Isotopic technics recently refined to measure hepatic glyceride synthesis[9,247-249] are more likely to reveal the nature of the abnormalities in the presence of conventional diets. A combination of such technics with appropriate measurements of glucose turnover, plasma and tissue insulin activities, hepatic arteriovenous differences of glyceride and perhaps opportunity to measure hepatic glyceride and lipoprotein synthesis directly may be required to understand the abnormal "diversion" of substrate to glycerides that seems certain to underlie Type IV in some patients.

Decreased glyceride removal. The second possible abnormality in Type IV is a decrease in the ability of the adipose tissue to clear glyceride that is entering plasma at the normal rate. Two major stages at which abnormality might occur were discussed earlier. A deficiency of lipase activity at the capillary wall or in the cell is one, and defective glyceride resynthesis in the cell is the other. Although PHLA levels tend to go down on high-carbohydrate diets they are usually normal in patients with florid endogenous hyperlipemia on regular diets.[184] Granting the oblique nature of available assays, a deficiency in lipoprotein lipase activity is probably not the explanation for most of Type IV. Deficiencies of PHLA in diabetes[140,176] and pancreatitis[179] are perhaps more usually accompanied by mixed hyperlipemia, discussed below under Type V. Direct and correlative measurements of the capacity of adipose tissue to convert fatty acids to glyceride are not simple in man and have not been applied to the problem of hyperlipemia. Normal glucose metabolism is essential in this process, and the abnormalities of glucose tolerance in Type IV call attention to this site. Of particular interest is the question of whether insulin activity at the level of the adipose-tissue cell may not be abnormal.

Briefly noted as a third possible abnormality in Type IV is the formation of anomalous lipoproteins, resistant to normal processes of removal. Possible examples of this in Type IV are presently limited to certain dysglobulinemias described below.

Secondary Type IV

Type IV hyperlipoproteinemia is often secondary to other known diseases. In the differential diagnosis 2 disorders outstrip all others in frequency and in confusion, for it is sometimes very difficult to determine whether the hyperlipoproteinemia or the associated disease is the underlying abnormality. These conditions are diabetes mellitus and pancreatitis.

Diabetes mellitus.[64] As a rule, diabetes that is insulin-sensitive and generally occurring in younger nonobese subjects inclined to have ketosis is considered the *cause*, and the Type IV hyperlipoproteinemia developing when control is poor the transient *resultant*. In severe uncontrolled diabetes hyperlipemia is almost universal and often accompanied by eruptive xanthomas (xanthoma diabeticorum) and the other clinical features of any hyperlipemia. Adipose-tissue stores are briskly mobilized, plasma free fatty acid concentrations are high, and hyperlipemia develops soon after ketosis. Patients with insulin-dependent diabetes may have Type IV patterns or, when exogenous hyperlipemia is superimposed on the endogenous, Type V. Hyperlipemia clears rapidly when the diabetes is brought under control.

By contrast exogenous insulin has little effect on Type IV patterns or carbohydrate intolerance in the patients with mild diabetes in whom ketosis never develops. Commonest in the obese and middle-aged, the hyperlipoproteinemia responds to caloric restriction and sometimes to orally administered hypoglycemic agents.[250,251] The abnormal mechanism is unlikely to be related to excessive transport of free fatty acids.

Pancreatitis and alcoholism.[177-180,252-254] The combination of hyperlipemia and abdominal pain has been known for nearly half a century.[255] Probably the commonest cause is pancreatitis, not infrequently in alcoholism. In a few subjects like the patients with the Type I pattern already discussed, hyperlipemia apparently antedated the pancreatitis; in the majority pancreatitis apparently preceded the hyperlipemia. In acute pancreatitis lipoprotein patterns have been described that resemble Types I, IV and V.[179,254] For the chronic hyperlipemia persisting after an attack, Type IV is perhaps most common, but the existing data are too skimpy to support a generalization. Lipoprotein patterns are therefore of little help to the physician in the immediate dilemma posed by a patient with abdominal pain and hyperlipemia that are both of uncommon severity. Several suggestions are offered for management: to stop all fat intake until the pain is gone; to check for the chemical signs of pancreatitis; regardless of the outcome of these tests, to be cautious about undertaking any exploratory surgery; and when the patient has recovered, not to neglect to seek evidence of familial hyperlipemia in his relatives. No mechanism has been proved for the hyperlipemia in pancreatitis.

Alcohol.[180,256] Oral and intravenous administration of ethanol has been shown to cause endogenous hyperglyceridemia, but the plasma glyceride concentrations are less than those frequently seen in pancreatitis. The mechanisms whereby alcohol produces hyperlipemia are still being debated.[180] In most patients with glyceride levels in excess of 1000 mg. per 100 ml. there is chemical evidence of hepatocellular damage as well as pancreatitis. Low PHLA has been reported in alcoholism.[257] Zieve[178] has described the occurrence of hemolytic anemia and hyperlipemia in alcoholism. This association could be due to 2 distinct effects of alcohol since anemia and hyperlipemia may occur singly in chronic alcoholism. There is a tendency to be empirical about intake of alcohol in patients with Type IV. Hyperlipemia observed after a "binge" usually cannot be reproduced under experimental conditions, and the association is difficult to prove.

Glycogen-storage disease. Mild to severe hyperlipemia is frequently found in several types of glycogen-storage diseases.[64,258] We have examined plasma from 4 patients with glycogenosis (Cori Type I); the Type IV pattern was present in each.

Idiopathic hypercalcemia. Hyperlipidemia[109] or hypercholesterolemia[259] has been reported to be a frequent feature of idiopathic hypercalcemia of infancy; changes in lipoproteins have been reported in only a few cases.[109] The Type IV pattern has been observed in 2 children (studied in collaboration with Dr. Sidney Levin). In 1, it was possible to demonstrate that hyperlipemia was associated with exaggerated carbohydrate induction. It is not known whether the children who have recovered from this disorder, which may be associated with premature atherosclerosis, have persistent lipoprotein abnormalities.

Hypothyroidism. Thyroid deficiency should always be considered in a patient with the Type IV pattern.[260] It can occur, but is less common than the Type II anomaly, as previously described. The relation can only be proved by therapeutic trial.

Nephrotic syndrome.[27,64,261] The nephrotic syndrome can provide a panoply of lipoprotein patterns, ranging from a discrete increase in β lipoproteins to tremendous increases in pre-β lipoproteins.[100,261,262] The severity is related to the degree of hypoalbuminemia. The mechanism by which lipoprotein concentrations are increased in nephrosis has been much studied but not established. Suggestions have included a block in the "conversion" of pre-β lipoproteins to β lipoproteins[100] (a theoretical mechanism, also discussed under Type III) possibly associated with a decreased uptake of triglyceride by the adipose tissue.[27] Others have suggested that the hyperglyceridemia is secondary to an increased outflow of free fatty acids from adipose tissue[27] or that the proteinuria provokes increased hepatic synthesis of proteins (and lipoproteins).[261]

Dysglobulinemia.[263-270] One of the diagnoses often suggested by the sudden appearance of severe hyperlipoproteinemia is the presence of an abnormal plasma gamma globulin as in myeloma, cryoglobulinemia or macroglobulinemia. There have been an increasing number of reports of multiple myeloma associated with Type II or Type IV pattern. Xanthomas, tendinous, tuberous or eruptive in form, have sometimes been present. Xanthomatosis has been reported in multiple myeloma without definite hyperlipoproteinemia. The myeloma protein has usually been a β_{2A} globulin. Usually, there is a direct relation between the amount of abnormal protein and the degree of hyperlipoproteinemia. It has been variously considered that the abnormal protein itself serves as a lipid carrier, is complexed with β or pre-β lipoproteins in such a way as to retard their catabolism or serves as a cryoprecipitin for the lipoproteins.

Gestational hormones. Women who are taking progesterone analogues by mouth, alone or in combination with estrogens, for contraception or other purposes frequently have Type IV patterns.[271] The same thing is true of pregnancy.[272]

Other disorders. We have also observed Type IV patterns in association with gout, Niemann-Pick disease, Gaucher's disease, total lipoatrophy, Werner's syndrome and other diseases.

Primary Type IV

Doubtless, all the diseases of which Type IV may be only an accompaniment have not been distinguished. Neither have all the more subtle environmental facts that may cause sporadic appearance of the pattern in the population. Just as the incidence of primary forms of Type IV is not known, so is information meager about the number of such cases that are genetically determined. The limited experience at the Clinical Center appears to be the major source of data bearing on this last question.

In the two or three years in which phenotyping has become a more than casual activity, approximately 90 patients have been seen at the Clinical Center whose index patterns on regular diets could be classified as primary Type IV. This excludes many more in whom such a pattern has been detected in connection with collaborative studies, in analysis of pedigrees of propositi of other types or in other patients in whom obvious disorders causing secondary lipoprotein abnormalities could not be excluded. For 22 of the patients with Type IV patterns it has been possible to sample some of their parents, siblings and children. In only 5 kindreds have all parents and all sibs been available. The choice of the relatives sampled in all 22 kindreds was based solely on availability and is therefore presumably subject to only such bias as this might introduce. Familial occurrence has been detected in 15 of the kindreds. The data from this study offer some interesting insight into the familial distribution of Type IV.

Eighty-six parents, children or sibs of the 22 index patients with primary Type IV were sampled. Thirty-four (40 per cent) had Type IV patterns. If the "propositi" are added to the sample, the percentage of abnormal family members rises to 52 (56 in 108). The Type IV pattern was the only abnormal one observed in these kindreds. Both the mother and the father of 5 "propositi" were sampled. In 2 sets both were abnormal; 1 in each of 3 sets was normal, and the other had Type IV. Ten of a total of 21 parents sampled were abnormal. Of the sibs of the index patients 52 were sampled, and 21 (40 per cent) had Type IV. The remaining 13 relatives examined were some of the children of the propositi. Three of 13 had Type IV, and all the abnormal relatives were over twenty years of age. Of the 10 who were normal, 7 were still less than twenty years of age. These data are too incomplete to warrant any statistical evaluation or serious genetic interpretation. Proper controls are lacking, and doubtless more than one biochemical defect is represented. It does appear, however, that the incidence of the same lipoprotein abnormality is surprisingly high in relatives of patients with primary Type IV. "Vertical transmission" does occur, and only 1 parent may show the defect. The data expose a broad opportunity and great need for further population study.

Clinical features. The lipoprotein abnormalities in most of the familial examples of Type IV described above have been mild. A few cases have been quite severe. Examples have been described elsewhere in detail.[159] The clinical manifestations include those described above for Type IV in general. Glucose intolerance has been nearly universal, being present in 22 of 23 subjects tested so far in the overall familial sample. Some, but not all, of the familial examples have been associated with obesity. Subjects with the severest hyperlipemia have usually exceeded normal weight limits. Exaggerated carbohydrate inducibility has been present in 6 of 7 who were adequately tested.

Management. The treatment of primary Type IV presently includes weight control, avoidance of excessive dietary carbohydrate and hypolipemic agents, in that order. Obese patients with Type IV who are brought to ideal weight will have a dramatic improvement in lipoprotein patterns and, usually, in glucose tolerance. If excessive carbohydrate inducibility can actually be tested this is ideal; if not, it is reasonable to assume that most patients will have less hyperlipemia if they maintain ideal weight on a diet in which 45 to 50 per cent of calories come from fats, particularly of fairly unsaturated, low-cholesterol sources. This helps the patient keep down his craving for sugars and starches.

If the program outlined above does not lower plasma glycerides to below 400 mg. per 100 ml., drug therapy may be considered in addition. The orally administered antidiabetic agents may be of value[250,251] although they do not reduce lipoprotein patterns to normal levels in some patients. Hyperlipoproteinemia is no contraindication to their use in the management of accompanying diabetes. Certain agents promoting hypolipidemia were described in the discussion of Type II. The 2 most effective in decreasing hyperlipemia are chlorphenoxyisobutyric acid and nicotinic acid. The precautions mentioned earlier for use of these agents apply to Type IV as well. Heparin injections may temporarily reduce Type IV hyperlipoproteinemia.[222] Its chronic use in the treatment of hyperlipemia is not practical.

Type V Hyperlipoproteinemia[159]

Definition

There are some patients on a regular diet who have chylomicrons *and* increased amounts of pre-β lipoproteins in the usual postabsorptive sample. The Type V pattern has been reserved to designate this combination of exogenous and endogenous hyperlipemia. The legitimacy of Type V as an indicator of a specific disease, syndrome or group of disorders may be properly questioned. It may be, for example, merely a stage of Type IV, produced by sudden imposition of a heavy load of dietary fat on clearing mechanisms intolerably burdened by endogenous glycerides. This sequence has been experimentally demonstrated in more than 1 of the patients with Type IV referred to in the last section. A Type V

pattern may also appear when the severe exogenous hyperlipemia of Type I is clearing on the low-fat diets that cause modest carbohydrate induction in such patients. Type V could be a genotypic variant of Type IV, possibly in terms of homozygous expression of the same mutant (or mutants) or because of the interaction of other genes. Type IV patterns frequently occur in the few "Type V kindreds" sampled thus far. The significance of Type V, therefore, is not clear, but its recognition is important. It provides the therapist with a guide to dietary management and the experimentalist with a tool to determine the identity or separateness of clearing mechanisms for glycerides of different origin and the factors determining the rate of removal.

The incidence of patients with the Type V pattern on normal diets is not known. Twenty-one such patients have been screened at the Clinical Center. A partial sampling of the relatives of these has brought the total number of examples of Type V to 27. Our sampling of patients with acute diseases associated with hyperlipemia has been extremely limited, and the frequency of this pattern might be much greater in general hospitals.

There is no body of useful information about Type V in the medical literature to warrant a drawing of historical perspectives. Many examples of this abnormality have undoubtedly been reported, but it is only with the increasing use of polyvinylpyrrolidone gradients and starch-block and paper electrophoresis that the identification of mixed hyperlipemia has been sufficiently good to permit the study of patients with Type V patterns.

Lipoprotein Pattern

As shown in Figure 13, the Type V pattern is complex. Cholesterol and glyceride concentrations are elevated but in proportions too variable to be helpful in diagnosis. Simple observation of the plasma after it has sat overnight in the cold can be useful. As they do in Type I, chylomicrons appear as a cream layer at the top. However, the infranatant layer in Type V remains lactescent. In the polyvinylpyrrolidone gradient tube, top, intermediate and bottom layers of turbid particles are usually seen. On starch-block electrophoresis primary and secondary particles are accompanied by endogenous particles of α mobility.[6] On the paper electrophoretogram the pre-β lipoproteins usually leave their trail of endogenous glyceride from the origin, but the chylomicron band is identifiable as a discrete band at the point of application.[7] When the chylomicrons are few, or when both bands are very intense, the distinction can be hard and requires experience. Concentrations of α and β lipoprotein tend to be low. If precipitation technics are used, there is an increase in "low-density lipoproteins," but reliable distinction of chylomicrons from endogenous particles requires ancillary analyses.

Clinical Forms and Features

The patients with Type V patterns studied so far

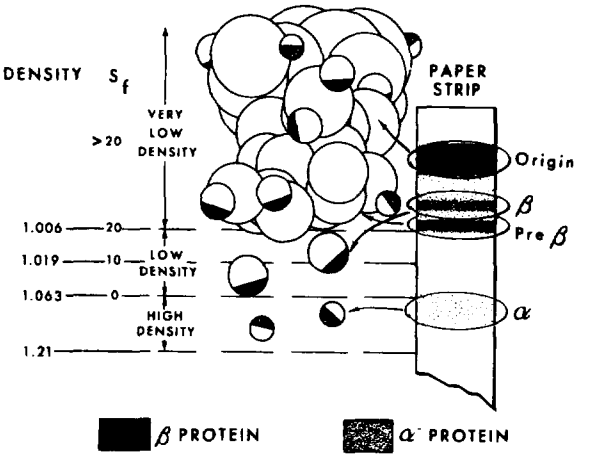

FIGURE 13. *Type V Hyperlipoproteinemia (See Also Table 3), Which Is a Mixture of Endogenous and Exogenous Hyperlipemia Uncertainly Related to Type IV.*

PHLA is normal or low; glucose tolerance is usually abnormal. Abdominal pain, eruptive xanthomas and hepatosplenomegaly are seen as in Types I and IV. The pattern is often familial, but there are "phenocopies" secondary to many disorders.

have many clinical features in common. Both males and females are affected. Symptoms have frequently appeared first in the late teens or the third decade. The commonest complaint is recurrent abdominal pain; this has often led to exploratory surgery, which has provided no specific diagnosis in most cases. Occasionally, the surgeons have found milky fluid in the peritoneal cavity. Sometimes, the appearance of eruptive xanthomas has been the first abnormality noted by the patient. As in Type I, these are commonly on the knees, buttocks, shoulders and back, but they may be anywhere. Lipemia retinalis, foam cells in the bone marrow and hepatosplenomegaly may be present. The patients are often obese and frequently have a family history of both diabetes and obesity. Evidence of accelerated atherosclerosis in either patients or their families has not been striking. Hyperuricemia may or may not be present. Pancreatic lipase and amylase may not be elevated even during bouts of abdominal pain. Glucose tolerance is almost invariably abnormal.

The contrast with Type I is important. Patients with Type I usually have symptoms beginning in infancy. There is no relation of their hyperlipemia to obesity or diabetes. The levels of PHLA in Type I are distinctly reduced. In Type V they may be somewhat low but are usually in the range of normal. The fall in hyperlipemia with a fat-free diet is usually rapid in both Types I and V, but the glyceride level is much more dependent upon the dietary fat intake in Type I. Type V hyperlipemia is much more affected by negative caloric balance. For both Types I and V one must be very cautious in refeeding fat after the patient has been on a fat-free, high-carbohydrate diet. Marked chylomicronemia and severe abdominal pain may develop in one day.

As with other types of hyperlipoproteinemia, Type V can be separated into primary and second-

ary forms, a distinction that for the present means simply the presence or absence of other diseases or conditions known to be associated with hyperlipemia. For example, the 21 index patients with Type V at the National Institutes of Health included 9 with what was considered to be primary and 12 with secondary. Of the latter, 3 had uncontrolled, insulin-dependent diabetes, and 5 chronic pancreatitis, and 4 were patients with history of recent heavy alcohol intake.[176] Full lipoprotein analyses have been done infrequently in cases of hyperlipemia due to alcoholic excess or pancreatitis, but many reports suggest that Type V patterns were present.

Genetic information. Of 9 "primary" examples of Type V in the Clinical Center group studied thus far (5 males and 4 females), all have proved to have at least 1 relative with hyperlipemia.

It has been possible to sample all the siblings of the propositi in 4 kindreds, and both parents of the propositi in 3. The parents of the fourth propositus are dead. All the 6 living parents had abnormal lipoprotein patterns. In 2 sets both had Type IV, and in the third, the father was Type V and the mother Type IV. The 4 propositi had 13 siblings. Four were normal; 3 had Type V, and 6 Type IV. Ten children of the propositi or their affected siblings were all normal, but none were over thirteen years of age. It is possible that the expression of the disorder is delayed, as was suggested in the discussion of Type IV, and young children should be treated separately in any population analyses until this point is clarified.

To sum up the lipoprotein patterns seen in 23 adult members of 4 kindreds of patients presenting Type V patterns, including the propositi, their siblings and parents, 8 had Type V, and 11 Type IV, and only 4 were normal. Not included in these analyses is another kindred only partially studied[159] in which at least 2 of 6 siblings have severe Type V patterns. The incidence of abnormal glucose tolerance tests in members of families with Type V and hyperlipoproteinemia (either Type IV or V patterns) is 16 of 17 subjects tested at the Clinical Center.

The sampling is so small as to make any genetic interpretations perilous. Nevertheless, it is worth comparing the data from the 4 completely sampled families with Type V just described with those obtained in relatives of propositi with Type IV described in the preceding sections.

The 4 propositi with Type V had 19 adult relatives (their sibs or parents) who were sampled. Four (20 per cent) were normal; 4 had Type V patterns, and 11 Type IV patterns. Fourteen patients who presented Type IV patterns and were then demonstrated to have primary familial hyperlipoproteinemia provided 54 adult sibs or parents for comparison. Twenty-four of these (44 per cent) were normal. All the 30 abnormal relatives were Type IV. The number of affected males and females was about equal. It is obvious that if 1 of the relatives with Type IV in the "Type V kindreds" had been seen first and had become the index patient, the distribution of patterns in the relatives of the 2 sets of propositi would have been different. This does not alter a strong impression from the available data that if the Type IV and Type V patterns represent the same genotype, such influences as determine 1 phenotype or the other seem not to be randomly distributed among all the affected families. With observation of the presence, so far, of abnormalities in both parents of the kindreds with Type V, one possibility is that Type IV and Type V are the heterozygous and homozygous genotypes, respectively, for identical mutant alleles. It is also possible that single genes for Type I and Type IV defects interact to produce Type V. The only tests of these and other possible explanations must come from the careful study of more kindreds. Very probably, the ultimate solution will require the development of better tests for genotype than these 2 lipoprotein patterns can provide.

Biochemical Mechanisms

Type V is a combination of abnormalities whose possible bases have already been discussed under Types I and IV. Speculation that the underlying lesion is comparable to that in Type IV, with the exogenous hyperlipemia a secondary feature attributable to diet, has already been mentioned. Acceptance of this theory will in part depend upon more rigorous proof than is available that chylomicrons and pre-β lipoproteins compete at identical sites for removal of their glyceride. Although this is probably so, more information will be needed to explain some of the family data just discussed.

If Type V represents the homozygous expression of Type IV one might expect that patients with V would be much more susceptible to carbohydrate induction. Adequate comparisons have not been made. If the mechanism in Type V is related to that in Type I, one would expect lipoprotein lipase deficiency to be present as detected by assay of PHLA. In 7 patients with Type V so tested the activity has varied from a low value of 0.17 in 1 patient to others clearly in the normal range during intake of regular diets. The test lacks the ability to discriminate clearly partial defects in enzyme activity as is evident in subjects assumed to be heterozygous for Type I. Tests are in progress to determine whether patients with Type V might have exaggerated de-induction of PHLA during low-fat feeding or retarded adaptation of the enzyme levels to the challenge of higher loads of dietary fat.

It has been reported that patients who have insulin-deficient diabetes, and Type V-like patterns when they are out of control, also have low PHLA activity that returns to normal when insulin is readministered and clearing of hyperlipemia occurs.[176] Patients with hyperlipemia and pancreatitis have also been reported to have decreased PHLA.[179] Diabetic animals clear ingested or infused glycerides

better when treated with insulin,[273] and a similar phenomenon has been shown in man.[176,274]

Management

If one may generalize about so elusive a syndrome, Type V appears to combine the hazards of Type I and Type IV. The treatment of Type V, beyond dietary manipulation, has not been systematically developed. The secondary and primary forms may be approached similarly from the standpoint of diet. Weight reduction in the obese patient is an excellent way to decrease hyperlipemia. As in Type IV, Type V patterns that return completely to normal levels on severe caloric restriction again become abnormal when isocaloric feeding is resumed at ideal weight. The hyperlipoproteinemia is usually much improved, however. Diets that are not unusually high in fats or carbohydrates should be used in maintenance. The patients must be followed carefully, both to forestall gain in weight and to discourage intemperance in alcohol, as well as to observe the lipoprotein pattern for unanticipated changes. Whenever severe endogenous hyperlipemia develops, care must be taken to reduce both calories and carbohydrate until improvement results, for a sudden switch to isocaloric high-fat diets may cause an abdominal crisis. The use of drugs has not been carefully described for Type V. The same considerations as mentioned under Type IV obtain.

Other Abnormal Lipoprotein Patterns

With the conclusion of the discussion of 5 types of lipoprotein patterns seen in patients with hyperlipoproteinemia, it will be clear to observers of other patients that some significant and perhaps diagnostic patterns have been left out of the numbering scheme. One may draw upon the experience with blood-clotting factors for encouragement to limit the entries in any numbered system. New patterns will inevitably be discovered as representative of other syndromes, and better technics will fragment the existing Types I through V. The nomenclature will evolve as it must. Nothing has been said of chemical abnormalities in the constituent lipids of the lipoproteins, such as the quantities of phytanic acid that appear in Refsum's syndrome[275] or small increases in cerebrosides in Gaucher's disease.[276]

Some other specific lipoprotein patterns such as the absence or deficiency of α and β lipoproteins have already been discussed in this review. One other set of patterns encountered quite often remains to be described. These are the patterns in certain liver diseases.

Obstructive Liver Disease

The lipoprotein patterns and serum lipids in patients with either intrahepatic or extrahepatic biliary obstruction are unique. They are characterized by marked hypercholesterolemia and hyperphospholipidemia. The increase in serum cholesterol is mainly confined to free sterol whereas the esterified cholesterol content is normal or low. The increase of phospholipid is relatively greater than that of the cholesterol. More often than not the serum triglyceride and free fatty acid concentrations are normal, and the plasma is icteric but perfectly clear.[277,278] The content of high-density lipoproteins is severely reduced or even absent in analyses made by preparative or analytical ultracentrifugation.[26,64] There is, on the other hand, a marked increase in the lipoproteins having density between 1.019 and 1.063. Comparison of the ultracentrifugal data to early work with Cohn fractionation pointed up a seeming paradox.[278] Usually, large amounts of lipid were present in the fraction IV that contains α lipoprotein in normal serum. The increased low-density lipoproteins were also shown to be abnormal, having lower than normal ratios of protein to lipid and cholesterol to phospholipid. They also did not react to antiserums specific for β lipoproteins.[278]

We have recently found that patients with biliary cirrhosis may have tremendous increases in α lipoproteins on the electrophoretogram and by immunochemical analyses. The α lipoproteins behave as though antigenically identical to the normal lipoproteins but migrate much more slowly during paper electrophoresis. These α lipoproteins, which contain more phospholipid than normal, float in the density region 1.006 to 1.063 and swell the concentration of low-density lipoprotein. Other patients with biliary-tract obstruction may have an elevation in true β lipoprotein that also contains more phospholipid than usual. The relation of bile stasis to these abnormal lipoproteins is unclear. It has been suggested that the overall lipoprotein increase may be related to the surfactant properties of bile salts favoring disruption of lipid-protein complexes and promoting a different type of stabilization of the lipids in plasma.[277] The liver probably also releases a good deal more lipid into the plasma because the normal flow of phospholipids and cholesterol in the bile is obstructed and because interruption of the enterohepatic cycle of cholesterol has altered the synthesis of lipoproteins. The hyperlipoproteinemia does vary with the extent and degree of biliary obstruction. The drug cholestyramine has been variably successful in lowering the hyperlipoproteinemia of biliary obstruction.[279] When severe hepatocellular damage occurs in the liver there is a lowering of all lipoprotein fractions, and patients with parenchymal liver disease may have severe total deficiency of α lipoproteins.[78]

Conclusions

The plasma is often the only window from which one can see the state of intracellular metabolism. The view is limited, and all ingenuity is demanded to gain the sharpest perspectives. The plasma lipoproteins have been discussed, and the vast information about them distilled from the point of view of the clinician who seeks the most rational approach

to patients with certain abnormalities in fat metabolism. The lipoproteins serve this purpose better than chemical determination of plasma lipid concentrations alone. It is hoped that the systematic way of using some of the simpler tools for lipoprotein analyses described will encourage wider application of lipoprotein patterns in clinical practice so that more specific diagnoses will replace certain of the time-worn clichés and more attention will be focused on the many basic problems of etiology that are still to be solved. Major emphasis has been placed on the use of lipoprotein patterns to seek genetic factors that produce altered plasma lipid levels. It has been one of the areas most neglected. If a pedigree chart appeared on the clinical record of every patient with hyperlipidemia, the number of unanswered questions might be reduced by half; certainly, the satisfaction that the physician gains from the study of patients with hyperlipidemia would be doubled.

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